IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

BOARD OF PATENT APPEALS AND INTERFERENCES

In re application of:

Group Art Unit: 1641

Serial No.: 08/886,044

Examiner: S. Devi

Filing Date: June 30, 1997

For: VACCINE AGAINST GRAM-NEGATIVE BACTERIAL INFECTIONS

FOURTH REVISED BRIEF ON APPEAL

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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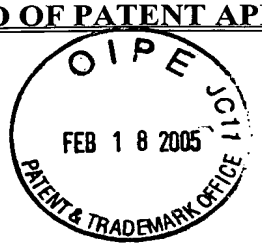

Attorney Docket No. 071007/0137

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APPELLANTS' FOURTH REVISED BRIEF UNDER 37 CFR §1.192

Commissioner of Patents
Customer Service Window
Randolph Building
401 Dulany Street
Alexandria, VA 22314

Sir:

This revised brief is filed in view of a telephone conference initiated by Examiner Devi on February 15, 2005. According to "BPAI FAQ – Rules of Practice before the BPAI" (http://www.uspto.gov/web/offices/dcom/bpai/fr2004/bpaifaq.html; copy attached), "an amended brief, based on an appeal brief originally filed prior to September 13, 2004, [is]...acceptable if it complies with either former §1.192 or §41.37(c)" of Title 37, CFR. The present brief complies with 37 CFR §1.192. The fees required under 37 CFR §1.17(f) were included in our Check No. 4716, filed with the initial brief on July 24, 2000. Please charge any fee deficiency or credit any overpayment to Deposit Account 19-0741.

REAL PARTY IN INTEREST

The real party in interest in this case is the Government of the United States, as represented by the Secretary of the Army.

1. RELATED APPEALS AND INTERFERENCES

There are no appeals or interferences, which will directly affect, be affected by, or have a bearing on, the Board's decision in this case.

2. *STATUS OF CLAIMS*

Pending: Claims 1-3, 5-8, and 15-17

Canceled: Claims 4, 9-14, and 18-20

Rejected: Claims 1-3, 5-8 and 15-17

Appealed: Claims 1-3, 5-8, and 15-17

3. *STATUS OF AMENDMENTS*

Two responses were submitted following final rejection. The first response was submitted on January 19, 2000, and cancelled claims 19 and 20. An Advisory Action dated March 15, 2000, indicated that the examiner had considered this response, and that for purpose of appeals, claims 1-3, 5-8 and 15-17 were pending and rejected. A second response was submitted on April 18, 2000, and canceled claims 12-14, 19 and 20. An Advisory Action dated June 1, 2000, indicated that the examiner had considered this response as well, and that it had been entered into the record for appeal. The claim status above reflects these amendments.

4. *BACKGROUND AND SUMMARY OF THE INVENTION*

The present invention relates to a vaccine effective against infections with Gram-negative bacteria and lipopolysaccharide ("LPS")-mediated pathology induced by Gram-negative bacterial infections. More particularly, it relates to a non-covalent, polyvalent complex vaccine containing purified *E. coli* LPS endotoxin and purified *N. meningitidis* outer membrane protein, which vaccine produces, in an actively immunized subject, an immune response against Gram-negative bacterial infection and the pathology caused by the LPS endotoxin.[1]

Infections by Gram-negative bacteria and consequent septic shock are leading causes of death among hospitalized patients. It is estimated that Gram-negative sepsis has an incidence of 70,000 to 300,000 cases per year in the United States. McCabe *et al., Am. J. of Med.* 68: 344 (1980). While attempts have been made to produce vaccines that will produce anti-endotoxin antibodies, and thereby protect against septic shock, results have been

[1] Specification at page 1, lines 8-17.

disappointing. For reviews, see Cross *et al., J. Endotox. Res.* 3: 57 (1994) and Greisman and Johnston, *J. Endotox. Res.* 4: 123 (1997).[2]

Because of an unmet need of long-standing for a vaccine effective against Gram-negative bacterial infections, the present inventors have devised a novel vaccine which allows for both active and passive immunization against Gram-negative bacterial infections. The present invention provides a vaccine, effective in actively immunizing a subject against infection by heterologous Gram-negative bacteria or against lipopolysaccharide (LPS) endotoxin-mediated pathology by the production of an antibody, comprising a non-covalent complex between (i) purified, detoxified LPS endotoxin derived from *E. coli* J5 strain and (ii) a purified outer membrane protein (OMP) derived from *N. meningitidis.*[3] The *E. coli* J5 strain is a so-called rough mutant strain of *E. coli* which produces LPS that lacks O-side chains.[4] The present invention also provides a method of actively immunizing a subject against infection by heterologous Gram-negative bacteria and LPS endotoxin-induced pathology, comprising administering to said subject an effective amount of a vaccine according to the invention.[5]

5. ISSUES

The sole issue on appeal in this case is whether claims 1-3, 5-8 and 15-17 would have been obvious based on Zollinger *et al.* (U.S. 4,707,543) in view of Ziegler *et al.* (*New Engl. J. Med.* 1982) or Myers *et al.* (U.S. 4,912,094) and Munford *et al.* (U.S. 4,929,604).

6. GROUPS OF CLAIMS

For purposes of this appeal, the claims do not all stand or fall together, but will be argued separately according to the following groups:

Group I Claims 1-3, 5, and 15-17

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[2] Specification at page 1 line 23 to page 2, line 1; Greisman and Johnston (1997) was provided and first discussed in response dated January 14, 1999.

[3] See claim 1, appended.

[4] See, for example, Ziegler or Calandra, of record. Both Calandra's work and Ziegler's work also are discussed in the specification on page 2, lines 1-9 and 19-21.

[5] See claim 6, appended.

Group II Claims 6-8 (see pages 22-23 of the Brief)

7. *SUMMARY OF THE ARGUMENT*

As elaborated below, the examiner has misapplied the primary reference, because it is not true that "Zollinger *et al.* teach the use of detoxified LPS from *Escherichia coli*, non-covalently complexed with OMP of group B *Neisseria meningitidis*, as a vaccine against infection." In the first instance, substitution of J5 LPS for the generic LPS in Zollinger must be based on an assumption that J5 will behave equivalently in terms of solubilizing OMP, an assumption that is not supported by the teaching in Zollinger. In addition, the underlying basis for the rejection based on Zollinger, that any combination of polysaccharide and OMP will produce equivalent results in a vaccine, has been conclusively rebutted by evidence in the record. And even if the essence of Zollinger were to combine LPS from *E. coli* and OMP from *N. meningitidis*, one skilled in the art would not have been motivated, by "the expected benefit of using an immunogen that elicits protective response against multiple pathogenic bacterial species," to substitute J5 LPS of Ziegler in Zollinger's complex, and there would have been "no reasonable expectation of success in using such a composition in active or passive immunizations against Gram negative bacterial sepsis." Moreover, the examiner has relied on teachings from Munford and Myers, to the effect that the structure of the R core region of LPS "is similar in most gram negative bacteria," despite appellants' demonstration that these teachings were debunked in the art when the present application was filed. The fact remains that, notwithstanding the widespread belief that J5 LPS is not an effective immunogen, appellants have succeeded in providing the key to unlock J5 LPS's latent immunogenicity.

8. *ARGUMENT*

The examiner's stated rejection

The claims in this case are rejected, under Section 103(a), over the combination of Zollinger in view of Ziegler or Myers and Munford. For a full expression of the basis for this rejection, it is necessary to return to the Official Action dated September 14, 1998. There the examiner urges that:

> Zollinger *et al.* teach the use of detoxified LPS obtained from *Escherichia coli*, non-covalently complexed with OMP of group B *Neisseria meningitidis* as a vaccine against infection...
>
> Ziegler *et al.* teach a purified LPS of *E. coli* J5 and its role as an effective immunogen.
>
> Myers *et al.* teach that "the core region is highly conserved among LPSs obtained from different genera of *Enterobacteriaceae* and that immunity against the core region is...protective against a wide variety of Gram negative bacterial challenges" and was "demonstrated by the work of Ziegler *et al*....
>
> Munford *et al.* teach that "the structure of the lipid A moiety is highly conserved' in the LPS of many pathogenic bacteria...that LPSs may be used as vaccines to prevent gram negative bacterial sepsis by producing antibodies to R-core regions....[and that] the structure of the R core region of LPS "is similar in most gram negative bacteria"...
>
> With this perspective, the examiner concludes that:
>
> It would have been obvious to one skilled in the art at the time the invention was made to substitute Zollinger's generic *Escherichia coli* LPS with its O-specific side chains intact, with Ziegler's or Myers' specific *E. coli* J5 LPS which is devoid of O-specific side chains, to produce the instant invention because, Zeigler *et al.* teach that O-specific side chains present in the LPS of parent *E. coli* strain 'conceals' the protective core determinants whereas *E. coli* LPS devoid of O-specific side chains has this protective core determinant exposed (and thus available for recognition by the host immune system). One skilled in the art would be motivated to produce the instant invention for the expected benefit of using an immunogen that elicits protective response against multiple pathogenic bacterial species in addition to *E. coli* (for example *S. typhimurium* and the meningococcus) because the exposed/unblocked conserved antigenic determinants that this immunogen presents to the host immune system as taught by Ziegler *et al.* or Myers *et al.* or Munford *et al.* One skilled in the art would have had a reasonable expectation of success in obtaining the vaccine of the instant invention since Ziegler's purified *E. coli* J5 LPS would be expected to function no differently than Zollinger's generic *E. coli* LPS when complexed with meningococcal outer membrane protein.

The most recent action expands on the examiner's rationale for combining OMP from *N. meningitidis* and J5 LPS from the *E. coli* J5 strain. In particular, she urges that the skilled practitioner would have been motivated "to substitute Zollinger's generic *E. coli* LPS with Zeigler's specific J5 LPS to produce the instant composition and vaccine for the expected benefit of economically and advantageously immunizing a subject against heterologous Gram negative bacterial sepsis with a single, all-in-one composition, with a reasonable expectation of success

in using such a composition in active or passive immunizations against Gram negative bacterial sepsis."

It is not true that "Zollinger et al. teach the use of detoxified LPS from Escherichia coli, non-covalently complexed with OMP of group B Neisseria meningitidis, as a vaccine against infection."

Zollinger discloses a "process for preparing a detoxified polysaccharide-outer membrane protein complex from bacterial envelopes. The so-obtained products which are useful against infection by the ***same*** bacteria" (abstract, emphasis added). The ***purpose*** of the polysaccharide in Zollinger, whether capsular polysaccharide or lipopolysaccharide, is to solubilize the outer membrane proteins.

Thus, Zollinger speaks of "outer membrane proteins...solubilized by the tetravalent mixture of A, C, Y, and W135 polysaccharides" (col. 2, lines 7-9). He also states that "the detoxified [lipopolysaccharide] was shown to retain its ability to bind to and ***solubilize*** outer membrane proteins" (col. 8, lines 66-68), and that "sonication is often essential to facilitate the protein-lipopolysaccharide interaction and ***solubilize*** the protein" (col. 9, lines 13-15; emphasis added in each case). For the purpose of solubilization, either detoxified lipopolysaccharide or capsular polysaccharide can be used, *i.e.* the teaching of Zollinger is that all lipopolysaccharides and capsular polysaccharides are equivalent. Zollinger teaches that the process in question is applicable generally to the preparation of detoxified polysaccharide-protein complexes derived from gram-negative bacteria, preferably *Neisseria meningitidis* group B, *Haemophilius influenze* type b, *N. gonorrhoeae*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

From the foregoing, it is apparent that the examiner has applied Zollinger in a selective manner, picking OMP from one species (*N. meningitidis*) and detoxified LPS from another (*E. coli*). ***This mixing and matching is not suggested by Zollinger, however.***

Indeed, Zollinger's only specific examples, one with capsular polysaccharide and another with detoxified lipopolysaccharide, entail the use of OMP from the ***same species*** as the polysaccharide. In his Example 1, outer membrane protein from *N. meningitidis* is complexed with capsular polysaccharides from serogroups A, C, Y, and W-135 of *N. meningitidis*. In Example 3, outer membrane protein from *N. meningitidis* is complexed with detoxified lipopolysaccharide from a serogroup B case of *N. meningitidis*.

The use of outer membrane protein and polysaccharide from the ***same species*** is consistent with another Zollinger teaching, that the vaccine is directed against the same species from which the OMP is obtained. If the polysaccharide is to fulfill any purpose in addition to the solubilization function taught by Zollinger, the clear indication in the reference is that it serve to strengthen the antigenic response to the OMP. This would best be achieved by using polysaccharide from the same species as the OMP.

Accordingly, the allegation that Zollinger teaches a combination of OMP from *N. meningitidis* and LPS from *E. coli* is a hindsight reconstruction of the art and, hence, should be withdrawn. Such an *N. meningitidis/E. coli* combination not only falls outside the ambit of Zollinger's teachings but also is contrary to Zollinger's express purpose.

Substitution of J5 LPS for the generic LPS in Zollinger must be based on an assumption that J5 will behave equivalently in terms of solubilizing OMP, an assumption that is not supported by the teaching in Zollinger.

Since Zollinger does not specifically teach the use of endotoxin derived from J5 mutant, the basis for the present rejection must be that it would have been obvious to substitute endotoxin from this mutant for capsular polysaccharides or for lipopolysaccharide purified from a serogroup B case strain because equivalent results would be achieved, *i.e.*, ***that the J5 LPS would be equally effective in solubilizing the outer membrane protein***. The premise on which the rejection is based must be that J5 lipopolysaccharide would be expected to behave equivalently in combination with outer membrane protein ***in terms of the ability to solubilize outer membrane protein, since that is the purpose of the LPS in Zollinger***.

Would LPS without O-chains be expected to solubilize outer membrane protein as effectively as LPS with O-chains? Zollinger did not use LPS without side chains, and so provides no direct guidance on this issue. However, Zollinger does specifically comment at the bottom of column 8 that "the detoxified product was shown to ***retain its ability to bind to and solubilize outer membrane proteins***" (emphasis added). That is, Zollinger felt it necessary to comment on whether a modification of LPS to remove part of it, *i.e.*, the Lipid A moiety, would affect its ability to achieve the stated purpose for the LPS in the Zollinger, namely the ability to bind to and solubilize outer membrane protein. This suggests uncertainty over whether an LPS molecule modified to remove Lipid A would retain the necessary solubilizing properties. A skilled artisan might doubt, as well, the ability of

LPS without the O-chains effectively to solubilize outer membrane protein, thereby undermining the alleged case of obviousness.

The underlying basis for the rejection based on Zollinger, that any combination of polysaccharide and OMP will produce equivalent results in a vaccine, has been conclusively rebutted by evidence in the record.

The examiner urges in her rejection that "Ziegler's purified *E. coli* J5 LPS would be expected to function ***no differently*** than Zollinger's generic *E. coli* LPS when complexed with meningococcal outer membrane protein" (emphasis added, citation above). Thus, the examiner urges equivalence as the basis for the substitution of J5 LPS for the lipopolysaccharide or capsular polysaccharide disclosed in Ziegler or Myers.

There is evidence in the record, however, that J5 LPS functions ***entirely differently*** than "Zollinger's generic *E. coli* LPS" when complexed with OMP. Forwarded with appellants' response submitted June 19, 1998, was a declaration by Dr. Cross, one of the co-inventors of the present claims. In the June 19th declaration, Dr. Cross discusses studies which compare combinations of OMP derived from *N. meningitidis* and purified, detoxified LPS endotoxin derived from *E. coli* strain J5 and combinations of OMP with purified, detoxified LPS endotoxins from other strains of *E. coli*.

The comparison in the June 19th declaration is a comparison to more than that which is shown in any concrete example in Zollinger. As noted above, the only concrete examples in Zollinger relate to combinations of OMP and LPS which both are from *N. meningitidis*. Thus, in Example 1, outer membrane protein from *N. meningitidis* is complexed with capsular polysaccharides from serogroups A, C, Y, and W-135 of *N. meningitidis*, while in Example 3, outer membrane protein from *N. meningitidis* is complexed with detoxified lipopolysaccharide from a serogroup B case of *N. meningitidis*. Appellants compare to a combination of OMP from *N. meningitidis* with LPS from *E. coli*, the combination alleged by the examiner to be generically encompassed by Zollinger. In other words, the comparison performed by appellants relates to the examiner's hindsight interpretation of Zollinger.

Experiments described in paragraphs 2-5 of the June 19th declaration examined the efficacy of active immunization with four different vaccines. A first group of mice was immunized only with *N. meningitidis* OMP, a second with *Brucella* LPS complexed to *N. meningitidis* OMP, a third with J5 LPS complexed to OMP, and a fourth with EC018 LPS

complexed to OMP. Vaccination with J5-OMP led to 90% survival, 50% greater protection than vaccination with EC018-OMP, a complex of OMP with another strain of *E. coli*. The results in the Cross declaration therefore show that combinations of OMP derived from *N. meningitidis* and purified, detoxified ***LPS endotoxin derived from E. coli strain J5*** provide unexpectedly superior protection against Gram-negative sepsis as compared to combinations of OMP with purified, detoxified ***LPS endotoxins from other strains of E. coli***. This is particularly surprising in view of the fact that J5-OMP vaccine was providing protection against a heterologous strain (EC018) whereas EC018-OMP was providing protection against the same strain.

These results could not have been predicted based on Zollinger, which teaches that capsular polysaccharides and wild-type lipopolysaccharides behave ***equivalently*** in combination with an outer membrane protein for the purpose of solubilization as disclosed in Zollinger. This evidence conclusively rebuts the examiner's allegation that "Ziegler's purified *E. coli* J5 LPS would be expected to function no differently than Zollinger's generic *E. coli* LPS when complexed with meningococcal outer membrane protein."

> ***One skilled in the art would not have been motivated, by "the expected benefit of using an immunogen that elicits protective response against multiple pathogenic bacterial species," to substitute J5 LPS of Ziegler in Zollinger's complex, and there would have been "no reasonable expectation of success in using such a composition in active or passive immunizations against Gram negative bacterial sepsis."***

It is the PTO's burden to find, within the cited art, evidence of motivation for the skilled artisan to have complexed LPS from the J5 strain, instead of detoxified LPS or capsular polysaccharide from other strains, with an outer membrane protein from N. meningitidis. Casting about for such evidence, the examiner argues that the J5 LPS would serve "as an immunogen to treat sepsis caused by multiple gram negative bacterial pathogens" based on Ziegler *et al.*

At an interview on February 14, 2000, however, Dr. Cross explained that all previous attempts to immunize or otherwise protect individuals against LPS endotoxin-mediated pathology had been unsuccessful. There is perhaps no better substantiation of this statement than a review article by Greisman and Johnston, published in 1997 (of record). Entitled "Evidence against the hypothesis that antibodies to the inner core of lipopolysaccharides in antisera raised by immunization with enterobacterial deep-rough

mutants confer broad-spectrum protection during Gram-negative bacterial sepsis," the Greisman/Johnston article is much more recent than any of the art cited by the examiner. Moreover, Dr. Greisman is a well-respected expert in this field and has received Honorary Lifetime Membership in the International Endotoxin Society, the highest award bestowed by that organization upon investigators involved in endotoxin research.

Included among the articles reviewed by Dr. Greisman is Ziegler *et al.*, *New England J. Med.* (1982), the only reference, presently recited, that relates to LPS from J5 mutants. The Greisman article also considers many of the prior-art attempts to achieve protection with antisera to LPS from J5 mutants. As to each and every study implicating broad-spectrum protection by rough-mutant antisera, Dr. Greisman concluded that defects, in design or methodology, had engendered inconsistent results, and that antisera to the J5 chemotype "do not appear capable of providing broad-spectrum protection."

The Ziegler article, cited by the examiner against the present claims, is discussed in the last paragraph on page 126 of Greisman *et al.* Greisman notes Ziegler's report of broad-spectrum protection with antisera to J5 rough mutants. In view of Ziegler's results, Dr. Greisman reevaluated the putative anti-LPS effect of rough mutant antisera, but first screened the antisera to preclude polyclonal increments in O-specific antibodies to the challenge LPS, and used pre-immune sera from the respective donors as controls.[6] When these steps were taken, Dr. Greisman found that rabbit antisera to J5 mutant, which possessed titers of antibody to the respective LPS core determinants comparable to or higher than those used by investigators who reported broad-spectrum protection against LPS, nevertheless failed to protect mice against lethality produced by LPS from heterologous smooth enterobacteria or even from the homologous smooth parental strain. According to Dr. Greisman, these results "failed to support the hypothesis that antisera to J5 and R595 are capable of effective broad-spectrum neutralization of the lethal activity of S-form LPS." *Id.* at page 127.

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[6] Also of record are the following articles by Dr. Greisman, relating to this reevaluation:

"Failure of Antisera to J5 and R595 Rough Mutants to Reduce Endotoxemic Lethality," *J. Inf. Dis.* 157:54-63 (1988), and

"Experimental Gram-Negative Bacterial Sepsis: Reevaluation of the Ability of Rough Mutant Antisera to Protect Mice," *Proc. Soc. Exp. Biol. Med.* 158:482-490 (1978).

Dr. Greisman's conclusion regarding Ziegler's study was shared by others in the field. Indeed, Ziegler herself was unable to identify antibodies as a basis for the protection observed ("we could not relate protection to the J5 antibody titer, regardless of the immune status of the donor" – page 1228, last paragraph). Zanetti *et al.* (of record) stated that, "as was noted in the report by Ziegler *et al.*, protection was related to immune plasma, not to specific levels of antibody to core LPS in a given plasma" (first paragraph on page 988) and, "as already noted, in both successful clinical studies with *E. coli* J5 antiserum, the protection remained of unclear origin because outcome could not be convincingly correlated with the level of antibodies to the core LPS of *E. coli* J5...the protection afforded by *E. coli* J5 antiserum could not be attributable to antibodies to the LPS of *E. coli* J5" (second full paragraph on page 988). Similarly, Glauser *et al.* (of record) noted that "a favorable outcome could not be correlated with antibody titers in either of the two clinical studies done with human polyclonal antisera to J5...the mechanisms of protection by antisera to J5 remain unknown" (second full paragraph on page S208). Baumgartner opined that "the successful studies did not discover the factor responsible for the postulated crossprotection in J5 antiserum, because the protection could not be attributed to anti-J5 LPS, anti-Re LPS, or anti-lipid A antibodies" (top of page 923).

In subsequent clinical trials, use of an anti-J5 LPS monoclonal antibody, rather than polyclonal antiserum from donors, fared no better in providing protection. Results of these trials were appended as Exhibit 2 in appellants' response filed April 18, 2000.

In the first of these, a trial by Ziegler *et al.* which was reported in the *New England Journal of Medicine* in 1991, the mortality rate among all patients with sepsis was 43 percent among recipients of placebo and 39 percent among those given HA-1A. Ziegler concluded that this result is similar to that obtained in their earlier trial with polyclonal antiserum. Two trials with HA-1A that were conducted by other researchers, however, produced different results. A trial reported by McCloskey *et al.* in 1994 in the *Annals of Internal Medicine* concluded that "HA-1A was not effective in reducing the 14-day mortality rate in patients with gram-negative bacteremia and septic shock." A trial in children with meningococcal septic shock, as reported by Derks *et al.* in a 1999 issue of *Clinical Infectious Diseases*, concluded that "no significant benefit of HA-1A could be demonstrated."

Dr. Greisman reviews results in five other clinical trials, in a declaration appended as Exhibit 4 to appellants' April 18th response. In his declaration, Dr. Greisman attests to failures of five clinical trials to show broad-spectrum protection during Gram-negative bacterial sepsis.[7] All of these trials were subsequent to Ziegler's clinical study of polyclonal antiserum, reported in 1982 and cited by the examiner.

In the first negative trial, performed by Braude and Ziegler's group, pre- and post-immune J5 antisera were given prophylactically to patients with neutropenia. The results, however, evidenced no differences in the rates of Gram-negative bacteremia, febrile episodes or mortality. In the second clinical study, a gamma globulin fraction from donors with elevated antibody titers to J5 LPS proved ineffective. In a third trial, an IgG fraction from sera of volunteers immunized with the J5 mutant provided no more protection against mortality than the IgG fraction from standard plasma pools, and did not reduce the number systemic complications of shock and did not delay the occurrence of death from systemic shock. A fourth clinical trial, in which infusions of human immunoglobulin preparations selected for their high content of IgG to R595 LPS, failed to show any greater protection against subsequent Gram-negative bacterial infections or their systemic complications in patients at high risk after major surgical procedures than was achieved with comparable immunoglobulin preparations containing on average 7-fold lower amounts of anti-R595 IgG. Finally, in a fifth clinical trial, 73 children with severe infectious purpura, the majority secondary to *N. meningitidis*, received J5 immune or pre-immune plasma. The anti-J5 plasma did not affect the clinical course, or the rate of decrease of TNFα or IL-6 or mortality.

In both his declaration and his review article, Dr. Greisman documents other attempts to demonstrate the protective capacity of J5 antisera in the laboratory. Many of the articles reviewed in Greisman *et al.* are the very ones listed by the examiner as showing the state of the art, including work by McCabe, Dunn, Young, Braude, Davis, Cryz, Di Padova, Nelles, Lugowski, and Salles. These articles have not been cited against the claims, and appellants will not discuss the flaws in each of them; to do so would risk obscuring the forest for the trees. Rather, appellants rely on the considerable expertise of Dr. Greisman in thoroughly reviewing the state of the art in this field, *circa* 1997. Dr. Greisman's review

[7] Copies of articles relating to each of these five clinical trials were appended as Exhibit 5 to the April 18th response.

reveals the flaws in each of these studies, which led to divergent results, and after his thorough review Dr. Greisman still believes that an anti-endotoxin vaccine for effective broad spectrum therapy of sepsis is unlikely to be developed.

Indeed, Dr. Greisman is not alone in his opinion. In March Dr. Cross attended a worldwide conference on sepsis in Europe. Over 1,000 papers were presented, yet only two dealt with antibodies against endotoxin, one by Dr. Cross and one on the WN1 monoclonal of Sandoz. Dr. Dunn, whose work in hyperimmunizing horses was included in the "state of the art" referenced by the examiner, was present. He was, however, silent on the possibility of an LPS vaccine.

In short, Dr. Greisman's article and declaration, as well as the other information discussed in this section effectively rebut the contention that a skilled artisan would reasonably have expected J5 LPS to act as an effective immunogen in a vaccine preparation to elicit a protective response against multiple pathogenic bacterial species, and more particularly against sepsis. Since antibody induced in response to J5 LPS alone does not provide effective protection against sepsis, the argument that it would have been obvious to complex LPS from a J5 strain with OMP from *N. meningitidis* instead of LPS from *N. meningitidis* "as an immunogen to treat sepsis" is totally without basis.

In an Advisory Action dated June 1, 2000, the examiner suggests that Dr. Greisman is a lone voice crying in the wilderness -- that he "fail[ed] to cite and/or discuss a plethora of positive studies, published in the art prior to the filing of the instant application."[8] There are others, however, who echo Greisman's sentiments on this subject. Like Greisman, a highly respected group in Switzerland led by Baumgartner and Glausner also has questioned the strength of the data presented in positive studies. An article by Baumgartner (1991), of record, reviews many unsuccessful clinical trials. Of the few successful trials reviewed, "the protection could not be attributed to anti-J5 LPS, anti-Re LPS or anti-lipid A antibodies" (page 923). And as recently as 1997, Zanetti and Glauser conclude that the "failure of these trials concluded three decades of research on anti-endotoxin approaches..."[9] And even an article on which Munford is a coauthor states that "proof that therapies

[8] Advisory Action at page 11.

[9] A copy of Glauser and Zanetti was appended to the Additional Evidence Submitted under MPEP 1207 filed February 18, 2004.

specifically targeting endotoxins work in human septic shock is still lacking."[10] So Greisman is not alone in his pessimistic views relating to prevention and treatment of sepsis.

One of the "positive" trials, alleged by the examiner to have been ignored by Greisman, is Cryz *et al.* The examiner argues that:

> Cryz *et al.* (*Eur. J. Clin. Microbiol.* 4:180-185, 1985) demonstrate, by active immunization, that an O-polysaccharide-deficient lipopolysaccharide derived from *E. coli* J5 LPS (administered in the absence of a strong adjuvant such as group B meningococcal OMP) is immunogenic in mice....Mice immunized with J5 LPS alone showed 70% protection against challenge with a heterologous Gram negative bacterium, such as *Pseudomonas aeruginosa* E576....this study provided the biological evidence of cross-reactivity and cross-protection, against a heterologous Gram negative pathogen, afforded by an unconjugated or non-complexed purified J5 LPS.[11]

Cryz *et al.* clearly state that anti-core antisera derived from J5 afforded substantial protection against only one of five *Pseudomonas* challenge strains. They conclude in their abstract that "attempts to demonstrate cross-serotype protection using O-antigen deficient and core deficient *Pseudomonas aeruginosa* lipopolysaccharide antigens was, for the most part, unsuccessful" (emphasis added). Amazingly, the examiner cites the data only on that one strain of *Pseudomonas* (shown in Table 2, p. 182), totally ignores the lack of protection on the other four, and completely ignores the authors' own conclusions. This is not only unfair to the authors, but evidences an inability on the part of the examiner to accurately characterize the disclosure of art that she has cited.

Moreover, Greisman did not ignore Cryz -- it is discussed on page 127 of the Greisman article. There it is noted that "Cryz *et al.* demonstrated that murine antisera to J5 LPA failed to cross-react in an ELISA with S-form LPS from three wild-type strains of *P. aeruginosa*."

Other "positive" studies listed by the examiner on pages 11-18 of the Advisory Action as being ignored by Greisman were, in fact, dealt with by Greisman. Thus, Greisman notes on page 126 that Wickstrom *et al.* (article K in the Action), demonstrated that although bovine J5 antiserum was more protective than saline against *E. coli* sepsis in calves, 'normal

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[10] Morrison *et al.*, *ASM News*, 60:479 (1994), appended to the Additional Evidence Submitted under MPEP 1207 filed January 8, 2004..

[11] Advisory Action at page 11-12.

serum was almost as helpful as immune [j5] serum'." The studies of Schwartzer *et al.* (article L in the Action) are characterized among references 35-52 on page 126 of Greisman as showing an "inability to demonstrate significant protection after either active immunization with rough-mutant bacterial vaccines of passive immunization with the resultant antisera." The examiner characterized Schwartzer as teaching immunization with a "safe, heat-killed *E. coli* J5 vaccine." This is, at best, an overly favorable characterization of Schwartzer's vaccine. Indeed, of 16 vaccinees, only 9 returned for a second inoculation. The other 7 refused a second inoculation, based on their adverse reactions after this first injection, which included pain, tenderness, induration, and erythema beginning within 6 hours of administration, as well as more severe reactions, including myalgia, low-grade fever, chills, sweats, abdominal cramps, nausea, diarrhea, and back pain. Generally, a vaccine with such a high incidence of such severe side effects would not be characterized as "safe." The examiner states that "in 50% of the vaccinees, a four-fold or greater response in anti-J5 LPS antibody response was mounted." She carefully ignores, however, Schwartzer's report that the response was transient and not increased by revaccination.

Appellants also have provided evidence that teachings from Munford and Myers, to the effect that the structure of the R core region of LPS "is similar in most gram negative bacteria," were debunked in the art when the present application was filed.

Myers is cited by the examiner as teaching that the core region is highly conserved. This fact has long been known. While there are highly conserved epitopes in the LPS core, however, others have shown that there exists a microheterogeneity in these epitopes. See, for example, Table 1 in the manuscript "Vaccines and Antibodies in the Prevention and Treatment of Sepsis" and Figure 11 of Lugowski (of record). In studies by Lugowski *et al.* (1996), in which core LPS from *E. coli* was used as a vaccine, there was no binding to *Klebsiella* (appellants' J5 LPS/OMP vaccine does bind to *Klebsiella*; see below). Moreover, there was little cross reaction between antiserum raised against the core LPS of J5 and other cores from *E. coli*, including the prototype core R3 to which J5 *E. coli* belongs! Thus, even within *E. coli*, there are significant differences between core epitopes.

In the Advisory Action dated June 1, 2000, the examiner responds that Figure 3 of Lugowski, *FEMS Immunology and Medical Microbiology* 16:21 (1996):

> convincingly demonstrates that, despite the stated structural microheterogeneity, an antiserum raised to *E. coli* R3 (J5) conjugate, in the absence of a strong adjuvant such as group B meningococcal OMP, cross-reacted well with the LPS of heterologous Gram negative bacteria including that of *Sh. flexneri, Citrobacter,* R2 core prototype of *E. coli, E. coli* 0111 serotype, and to some extent, also with the LPS of *Klebsiella pneumoniae, S. typhimurium* and Ra prototype of *E. coli.*[12]

Figure 3 of Lugowski shows antibody raised against the R3 core, the type of core seen in J5, but there is no way that Figure 3 shows clinically significant cross-reactivity. Figure 3 shows minimal, if any, binding to other cores of *E. coli*, and no binding to *Klebsiella* or *Salmonella*! Indeed, Lugowski concludes, on page 24, that the anti-R3 serum "shows the highest specificity among tested antisera. At low concentration it reacts only with R3 core type lipopolysaccharide and with *Citrobacter* 1487 LPS possessing closely related core type." (emphasis added). It is not understood how the examiner can possibly cite Figure 3 of Lugowski (1996) as suggesting cross-reactivity. Furthermore, "detectable" binding and the real binding indicative of a useful vaccine are quite different things. The very minimal degree of binding to E. *coli* that is disclosed in Lugowski would never prompt a skilled artisan to proceed with vaccine studies.

The examiner also notes in the Advisory Action that Lugowski (1996) was published after appellants' priority date. Appended to the Additional Evidence Submitted under MPEP 1207 filed January 8, 2004 is a paper by Munford published in 1980 in *J. Bacteriology* 144:630. The paper is entitled "Size heterogeneity of *Salmonella typhimurium* LPS in outer membranes and culture supernatant membrane fragments." Thus, even given Munford's later claims of homogeneity, he recognizes some heterogeneity. Another earlier article which mentions the heterogeneity of the core is Gibb *et al., J. Infect. Dis.* 166:1051 (1992), a copy of which was appended to Additional Evidence Submitted under MPEP 1207 filed January 8, 2004.. Gibb *et al.* examined 180 clinical isolates and found that 123 had an R1 core, 14 had an R2 core, 18 had an R3 core and 25 (14%) had none of these core types. This clearly shows the present of at least four core regions, with different patterns of reactivity. In addition to these four core regions, there exists microheterogeneity within each of the core regions, as previously discussed. For example, DiPadova was published before appellants' priority date and teaches that "microheterogeneity in the core structure is due to

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[12] Advisory Action at page 7.

nonstoichiometric substitutions with phosphate and ethanolamine groups."[13] All of these differences lead to a wide variation in core conformation.

In addition to the foregoing, appellants also appended to the Additional Evidence Submitted under MPEP 1207 filed January 8, 2004 an article by Ernst Rietschel, a highly respected LPS chemist. The article publishes data suggesting that it is the conformation of the LPS core, and not its linear structure, that is important in the interaction of LPS with mammalian hosts. In other words, even if cores possess only limited heterogeneity they may interact differently in the host, since even limited heterogeneity can give rise to differences in folding, and hence, conformation. Rietschel's theory supports appellants' hypothesis, *infra*, that the OMP serves to present an important conformational epitope.

DiPadova (of record) similarly suggests a conserved core region by disclosing that a monoclonal antibody binds to the 5 known cores of *E. coli* and to *Salmonella* core. DiPadova and colleagues generated their core LPS-specific monoclonal antibody by sequential immunization of animals with different LPS core structures. Even when animals were immunized with a variety of LPS core structures, however, the resulting monoclonal antibody had no activity against *Klebsiella* or *Pseudomonas*. (Appellants' J5 LPS/OMP vaccine does bind to *P. aeruginosa* and *Klebsiella*, as shown in the declaration dated January 12, 1999, that was submitted by Dr. Cross.) In the Advisory Action, the examiner attempts to rebut DiPadova along the same lines as Lugowski. Thus she notes, on page 19, that DiPadova "did show detectable binding to the LPS." It is noted, however, that the antibody bound only to some isolates of *Klebsiella* and to no isolates of *Pseudomonas*. It would not have been obvious to make "a vaccine, effective in actively immunizing a subject against infection by heterologous Gram-negative bacteria or against lipopolysaccharide (LPS) endotoxin-mediated pathology," based on this disclosure.

In a more recent editorial, published in 1992 and appended to appellants' April 18th response as Exhibit 6, Munford and colleagues state the premise on which Ziegler's trial was based, i.e., that the highly-conserved core region generates protective antibodies and is an effective immunogen, "remains unproved and unclear." In particular, they note that the

[13] DiPadove at page 3863, bottom of right-hand column.

results obtained in the preclinical studies of Ziegler antibody HA-1A were "substantially different from those more recently described." In addition, they cited information obtained under the Freedom of Information Act that "there is no experimental model in which HA-1A has consistently protected animals from endotoxic challenge." Open Meeting of the Vaccines and Related Biological Products Advisory Committee (September 4, 1991), Volume 1, Bethesda, Md.: FDA, 1991:50. Thus, subsequent to his patent filed in 1986, Munford found reason to suspect the premise that the highly conserved core region elicits effective protection.

Finally, Myers attaches significance to the core region based on the results obtained by Ziegler in her cited 1982 article, to wit: "...the core region is highly conserved among LPSs obtained from different genera of Enterobacteriaciae; immunity against the core region is therefore protective against a wide variety of gram negative bacterial challenges. This was demonstrated by the work of Ziegler *et al.*" (emphasis added). However, as explained in detail above, Ziegler's results were not due to antibodies against the core region. Ziegler herself was unable to correlate protection with J5 antibody titer, and many others of skill in the art have commented on this point. Accordingly, Myers' teaching adds nothing to that of Ziegler, based as it is on the faulty conclusion that Ziegler showed immunity against the core region.

In an attempted rebuttal of this point in the Advisory Action, the examiner states that the J5 LPS-induced antibodies in Ziegler "conferred protection against Shwartzman reactions caused by purified endotoxins from bacterial species as widely varied as *E. coli*, *Salmonella typhimurium*, and the meningococcus," citing page 1226 of Ziegler. This clever sleight of hand on the part of the examiner attempts to use a study in which antibodies were found to protect against Shwartzman reactions in animals to counteract Ziegler's failure to correlate protection to antibody titer in their human clinical study. Protection against Shwartzman reactions in an animal study, however, does not overcome the failure of Ziegler to correlate protection to antibody titer in their clinical trial.

In sum, the inferences drawn by the examiner based on teachings in Myers and Munford can be effectively rebutted. The teaching that the core region of LPS is conserved, in light of subsequent teachings highlighted above, would not have led a skilled artisan to conclude that the core region might provide the basis for protection against sepsis.

In spite of the widespread belief that J5 LPS is not an effective immunogen, appellants persisted and have succeeded in providing the key to unlock J5 LPS's latent immunogenicity

Yet appellants have succeeded where others have failed. They have done so by complexing the J5 LPS with OMP of *N. meningitidis*. This complexation with the OMP of *N. meningitidis* appears to maintain J5 LPS in a proper spatial configuration such that relevant cross-reactive epitopes in the J5 LPS core are exposed in a manner that they are not when simply conjugated to protein or given alone. A key aspect of appellants' vaccine is reflected in their demonstration that LPS of *E. coli* J5 (Rc chemotype) - the highly conserved core of endotoxin - can produce antibodies that provide protection against the biologic activities of heterologous LPS.

Proof that a vaccine of the present invention indeed is effective, in improving the outcome following a subsequent challenge with heterologous bacteria, is manifest in the data provided in Dr. Cross's declaration of January 12, 1999 (appended in Exhibit 1). That is, the Cross declaration documents studies of challenge with virulent strains of heterologous bacteria following active immunization with J5 LPS/OMP.

As described in the protocol appended to the declaration, rats rendered neutropenic with cyclophosphamide were immunized, either with de-O-acylated J5 LPS ("dLPS") complexed to OMP or with saline, in a 3-dose regimen prior to challenge with the heterologous bacteria. Levels of antibody titer for rats immunized with the J5 dLPS/OMP exceeded a target level of 800 ELISA units/ml of antibody, a level previously shown to be protective in passive protection experiments. Following immunization, the rats were challenged with either *Pseudomonas aeruginosa* or *Klebsiella pneumoniae*, in a dose which exceeded LD90 for this experimental model in previous studies.

The results showed that active immunization with J5 dLPS/OMP vaccine produced a prompt and sustained anti-core glycolipid antibody level that was generally in 100-fold excess of pre-immunization baseline levels. Twenty-four hours after bacteremia, antibody levels decreased, but then rapidly recovered to, and remained at, pre-infection levels. Active immunization with J5 LPS/OMP vaccine induced greater than 800 ELISA units/ml of antibody at the onset of neutropenia, nearly 4 weeks after the last dose of vaccine, and this level persisted throughout the entire period of neutropenia, for up to 80 days after the initial immunization. This is in distinct contrast to results achieved by passive immunization

with antibodies, where initial levels of 800 ELISA units/ml of antibody dropped to less than 200 ELISA units/ml of antibody by 24 hours. Thus, while levels of antibody produced in response to J5 LPS alone dropped to less than 200 ELISA units/ml of antibody by 24 hours, levels of 800 ELISA units/ml induced by immunization with LPS complexed with OMP from *N. meningitidis* are sustained for 80 days after immunization. This is clearly an unexpected result.

Immunization with J5 LPS/*N. meningitidis* OMP did not prevent either systemic infection or initiation of sepsis, but it clearly reduced the likelihood of a lethal outcome following infections with both heterologous strains of bacteria. Vaccinated animals challenged with *Pseudomonas* had an overall survival rate of 48% compared to 7% for saline treated control animals. A similar result ensued with *Klebsiella* challenge, with a 64% survival rate for vaccinated animals versus a 13% survival rate for control animals.

One particularly surprising result was the effect of the vaccine on organ colonization by the bacteria. Vaccinated animals had the same frequency and magnitude of bacteremia from the challenge strain as the control group, but had significantly lower levels of bacteria in liver and spleen than control animals.

In addition to the decreased bacterial colonization in liver and spleen, there was a significantly lower level of circulating endotoxin at the onset of fever in vaccinated animals as compared to control animals. While endotoxin levels increased in both groups at 24 hours, they were still lower than those of the control group. The lower level of circulating endotoxin may be due in part to promotion of LPS clearance from the circulation.

Antibodies produced in response to vaccination with appellants' vaccine do not appear to directly promote killing of bacteria, based on *in vitro* tests performed by appellants. They prevent neither systemic infection nor initiation of sepsis. They do, however, significantly reduce the likelihood of a lethal outcome following infections with both heterologous strains of bacteria. Dr. Cross hypothesizes that antibodies generated in response to the vaccine promote the uptake and killing of bacteria from the blood by tissue.[14]

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[14] Applicant herein clarifies and corrects an earlier statement that "Antibodies produced in response to vaccination with appellants' vaccine do not appear to promote killing of bacteria, either directly or indirectly."

Inexplicably, the examiner has given short shrift to these data. In an Official Action that is 21 pages long, the examiner devotes only six sentences to the issue of appellants' declaration. On page 10 of the Action, the examiner comments that

> Applicants submit a further declaration from Dr. Cross which provides results of challenge studies with heterologous bacteria. While the information in the declaration supports that provided in the specification, it does not overcome the rejection of instant claims under 35 U.S.C. 103(a).

No support for this statement is given in context. Much later, however, in the paragraph bridging pages 14 and 15 of the Action, the examiner explains that

> The data provided with Dr. Cross's declaration shows that administration of a vaccine-derived antiserum to animals results in 48% survival of animals against challenge with *Pseudomonas* and 64% survival against challenge with *Klebsiella*. However, 52% and 36% of immunized or treated animals respectively were not "protected." The full scope of the claims is not commensurate with the scope of the enabling disclosure and undue experimentation would be required by one of ordinary skill in the art to reproducibly practice the invention as claimed. The enablement (scope) provisions of 35 U.S.C. §112, first paragraph, are not met and the claim is viewed as non-enabled with respect to its scope.

This "scope" issue, raised in connection with the declaration, is neither advanced or elaborated in the balance of the Action. The only "scope" rejection propounded in the Action relates to claim 19, which was directed to passive, not active, protection. This point was discussed at the February 14th interview, and appellants understood Examiner Housel to agree that the scope of the claims was commensurate with both the disclosure and the scope of the showing. More particularly, the undersigned explained at the interview that claim 1, appellants' broadest vaccine claim, recites "a vaccine, effective in actively immunizing a subject against infection by heterologous Gram-negative bacteria or against lipopolysaccharide (LPS) endotoxin-mediated pathology by the production of an antibody, comprising a non-covalent complex between (i) purified, detoxified LPS endotoxin derived from *E. coli* J5 strain and (ii) a purified outer membrane protein (OMP) derived from *N. meningitidis*." Thus, the broad claim specifically recites both the immunogen and the immunocarrier, as well as the fact that they form a non-covalent complex. The specification clearly describes how to make and use this vaccine, and the data in Dr. Cross's declaration uses this same vaccine and shows positive results. Based on this, it is believed that no issue of scope remains following the interview.

The examiner's comments regarding the percentage of subjects that were protected, 48% and 64%, respectively, are not understood. There are other vaccines in common use that provide significantly less than 100% protection. For example, pneumococcal immunization is routinely used, and its efficacy is generally believed to be between 60 and 70% at best. A similar degree of efficacy is the case with influenza immunization. Clearly, vaccines that provide a percentage of protection similar to that demonstrated for the present vaccine are considered to have clinical value. It is submitted that the data in the second Cross declaration are in line with that for vaccines generally, and are sufficient to rebut any allegation with respect to the enablement or obviousness of the present invention. This point also was discussed at the interview and accepted.

Appellants now have obtained early results with their vaccine in human volunteers. These results were not submitted sooner because they just recently became available. The results are reported in a further declaration by Dr. Cross, which is appended to the Additional Evidence Submitted under MPEP 1207 filed concurrently herewith, and are in agreement with results obtained with the mouse model. Active immunization with the vaccine according to the present invention produced a 2- to 5-fold increase in the 24 volunteers following a 3 dose regimen. Moreover, all three doses of the vaccine were well-tolerated by all volunteers, as was a subsequent booster given at 8 months to 6 of the volunteers. This is in distinct contrast to the "safe" heat-killed vaccine of Schwartzer, which produced pain, tenderness, induration, and erythema beginning within 6 hours of administration, as well as more severe reactions, including myalgia, low-grade fever, chills, sweats, abdominal cramps, nausea, diarrhea, and back pain.[15]

While no prima facie case of obviousness exists with respect to any of the claims, the method claims, claims 6-8, present a different issue with respect to patentability.

Zollinger clearly disclose "products which are useful as vaccines against infection by the same bacteria, and method for protecting animals against the same infection" (abstract). All of the examples of Zollinger use outer membrane protein and polysaccharide from the same species, which is consistent with Zollinger's teaching that the vaccine is directed against the same species from which the OMP is obtained. Yet the examiner

[15] *Supra.*

maintains that it would have been obvious (1) to choose LPS from *E. coli* and OMP from *N. meningitidis* in Zollinger *et al.* and then (2) to substitute J5 LPS for the generic LPS of Zollinger *et al.* to produce a vaccine.

The foregoing arguments all address the lack of any teaching in the art both to select LPS from a different species than OMP in Zollinger and to substitute J5 LPS for the generic LPS of Zollinger. But even assuming, *arguendo*, that a skilled artisan might choose, for some reason not apparent in Zollinger, to use J5 LPS to solubilize the OMP of Zollinger, that still would not have suggested the *use* of that vaccine in a method of actively immunizing a subject against infection by heterologous Gram-negative bacteria and LPS endotoxin-induced pathology, as recited in claims 6-8. Zollinger does not relate to a method of immunizing a subject against heterologous Gram-negative infection or LPS endotoxin-induced pathology. Zollinger uses polysaccharide, whether capsular polysaccharide or lipopolysaccharide, to solubilize outer membrane proteins, and uses the combination to provide protection against LPS endotoxin-induced pathology and homologous, not heterologous, infection.

To modify Zollinger's method would be contrary to the express purpose of that method as disclosed in Zollinger, to provide protection from the "same bacteria." This is in clear contravention of MPEP §2143 ("the proposed modification cannot render the prior art unsatisfactory for its intended purpose"). If the polysaccharide is to fulfill any purpose in addition to the solubilization function taught by Zollinger, the clear indication in the reference is that it serve to strengthen the antigenic response to the *same* bacteria, *N. meningitidis*. This is best achieved by using polysaccharide from *N. meningitidis*. To use a different species of LPS, and to adminster a vaccine comprisng the same in a method of providing protection against heterologous infection and LPS-induced pathology, would be contrary to the stated purpose of the primary reference, which is improper.

9. CONCLUSION

From a reading of the Official Actions in this case, one would fairly expect that an effective endotoxin vaccine must have been approved long ago. This is especially so when one considers that nearly four hundred thousand cases of sepsis a year are documented, and sepsis is the leading cause of death in intensive care units. Clearly, there is a substantial

commercial market, and the search for a vaccine to prevent or alleviate the severity of sepsis has been an initial and major area of biotechnology's involvement in clinical medicine.

Yet, nearly a quarter of a century after the first publications cited by Examiner Devi, there still is no vaccine to prevent or alleviate the severity of sepsis. This highlights a basic inconsistency between what a skilled artisan would glean from the literature that is cited against appellants' claims and the reality of endotoxin vaccines. In the present case, the picking and choosing of pieces from various studies has led to an inaccurate conclusion regarding what would have been obvious in the field of endotoxin vaccines. The fact remains that no cited paper or any reasonable combination of cited papers provides the insight critical to a successful vaccine against sepsis, as presently claimed. The references lack the teaching necessary to produce a clinically useful vaccine.

For these reasons, the Board is respectfully requested to reverse the examiner and remand this application for issuance.

Respectfully submitted,

18 February 2005
Date

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APPENDIX: APPEALED CLAIMS

1. A vaccine, effective in actively immunizing a subject against infection by heterologous Gram-negative bacteria or against lipopolysaccharide (LPS) endotoxin-mediated pathology by the production of an antibody, comprising a non-covalent complex between (i) purified, detoxified LPS endotoxin derived from *E. coli* J5 strain and (ii) a purified outer membrane protein (OMP) derived from *N. meningitidis.*

2. A vaccine of claim 1, wherein said *E. coli* strain J5 is of the Rc chemotype.

3. A vaccine of claim 1, wherein said *N. meningitidis* is group B strain.

5. A vaccine of claim 1, wherein the weight ratio of said purified outer membrane protein to said purified and detoxified endotoxin in said non-covalent complex is between 1 and 2.

6. A method of actively immunizing a subject against infection by heterologous Gram-negative bacteria and LPS endotoxin-induced pathology, comprising administering to said subject an effective amount of a vaccine according to claim 1.

7. A method of claim 6, wherein said *E. coli* strain J5 is of the Rc chemotype.

8. A method of claim 6, wherein said *N. meningitidis* is group B strain.

15. An immunogenic composition comprised of the vaccine according to claim 1 in a pharmaceutically effective carrier.

16. A composition of claim 15, wherein said *E. coli* strain J5 is of the Rc chemotype.

17. A composition of claim 15, wherein said *N. meningitidis* is group B strain.

A. Effective Date (September 13, 2004)

Questions related to Time Periods for Filing of Papers:

A1. If a notice of appeal is filed before September 13, 2004, the effective date of the BPAI final rule, when is the appeal brief due?

If the notice of appeal is filed before September 13, 2004, the time period for filing an appeal brief will be the time period set forth in former § 1.192(a) which provides that the appellant must file an appeal brief: (1) within two months from the date of filing of the notice of appeal; or (2) within the time allowed for reply to the action from which the appeal was taken, if such time is later.

The time period set forth in former § 1.192(a) also applies if the notice of appeal is filed with a certificate of mailing or transmission in compliance with § 1.8 and the date on the certificate of mailing or transmission is before the effective date of September 13, 2004, but the notice of appeal is received by the Office on or after September 13, 2004. The two month time period will begin on the date of receipt of the notice of appeal.

A2. If appellant reinstates the appeal after the prosecution is reopened by filing a second notice of appeal on or after the effective date, when is the second appeal brief due?

Appellant must file the second appeal brief (in compliance with the format and content requirements of § 41.37(c)) within two months from the date of filing the second notice of appeal, even if the first notice of appeal and the first brief were filed before the effective date. The two month time period is extendable under the provisions of § 1.136 for patent applications and § 1.550(c) for *ex parte* reexamination proceedings. See § 41.37(e).

A3. If a notice of appeal is filed on or after the effective date of September 13, 2004, would extensions of time under § 1.136(a) be required when a Request for Continued Examination (RCE) under § 1.114 or an amendment is filed after two months from the date of filing the notice of appeal, but within three

months from the mailing of the action from which the appeal was taken?

Yes, extensions of time under § 1.136(a) are required for filing an RCE or amendment after two months from the filing of the notice of appeal, even if the RCE or amendment is filed within the three months from the mailing of the action from which the appeal was taken.

Questions related to Appeal Brief Contents or Requirements for Papers Filed after Appeal:

A4. If the notice of appeal is filed before the effective date of September 13, 2004 and the brief is filed by appellant on or after the effective date, would the appeal brief be required to comply with the content and format requirements of § 41.37(c)?

Yes, any appeal brief filed on or after September 13, 2004 must be in compliance with the requirements set forth in § 41.37(c) and be accompanied by the appropriate fee under § 41.20(b)(2). If the brief does not comply with § 41.37(c), an amended brief will be required under § 41.37(d).

Exception: If the appeal brief is filed with a certificate of mailing or transmission under § 1.8 and the date on the certificate of mailing or transmission is before September 13, 2004, the appeal brief may comply with either former § 1.192 or new § 41.37, even if the appeal brief is received by the Office on or after September 13, 2004.

A5. Would the Office accept an appeal brief filed before the effective date of September 13, 2004 that is in compliance with § 41.37(c)?

Yes, a brief filed before September 13, 2004 that is compliant with the new § 41.37(c) will be acceptable.

A6. If an appeal brief filed before the effective date of September 13, 2004 fails to comply with the content and format requirements of § 1.192 and the Office mails appellant a Notice that correction is required, would an amended appeal brief filed on or after the effective date be required to be in compliance with § 41.37(c)?

No, an amended appeal brief, based on an appeal brief originally filed prior to September 13, 2004, would be acceptable if it complies with either former § 1.192 or § 41.37(c), regardless of when the Office mailed a Notice requiring correction of the noncompliant appeal brief.

A7. If, after a final rejection or an appeal, applicant or appellant files an amendment, affidavit or other evidence on or after the effective date, will the revised or new rules in the BPAI Final Rule apply?

Any affidavit or other evidence filed after a final rejection, or an appeal, on or after the effective date, will be subject to the revised or new rules (*i.e.*, the revised § 1.116 or new § 41.33).

Questions related to Examiner's Answers and Supplemental Examiner's Answers:

A8. If the appeal brief is filed before the effective date of September 13, 2004, but the examiner's answer is mailed on or after the effective date, can the examiner's answer include a new ground of rejection?

Yes, an examiner's answer mailed on or after September 13, 2004 may include a new ground of rejection (with Technology Center Director or designee approval) in compliance with § 41.39. Any examiner's answer mailed before September 13, 2004, however, may not include a new ground of rejection. See former § 1.193.

A9. Can the examiner provide a supplemental examiner's answer under § 41.43 on or after the effective date of September 13, 2004 in response to any new issue raised in a reply brief that was filed before the effective date?

Yes, the examiner may provide a supplemental examiner's answer (with Technology Center Director or designee approval) if it is mailed on or after September 13, 2004 in response to any new issue raised in a reply brief, even if the reply brief was filed before September 13, 2004. Appellant may file another reply brief in compliance with § 41.41 to reply to the supplemental examiner's answer within two months from the date of mailing of the supplemental examiner's answer. Extensions of time under § 1.136(a) are not applicable to the two-month time period.

A10. If the Board remanded an application before the effective date of September 13, 2004 for further consideration of a rejection, and the examiner provides a supplemental examiner's answer on or after the effective date (in response to the remand by the Board), can appellant request that prosecution be reopened under § 41.50(a)(2)(i)?

No, appellant may **not** request that prosecution be reopened under § 41.50(a)(2)(i) in response to the supplemental examiner's answer since the Board remanded the application before the effective date. Appellant may request that prosecution be reopened in response to a supplemental examiner's answer written in response to the remand by the Board, only if: (1) the remand is on or after the effective date, and (2) the remand is for further consideration of a rejection. The Board should indicate in the remand if § 41.50 (a)(2)(i) applies. Thus, appellant may **not** request that prosecution be reopened under § 41.50(a)(2)(i) if the remand is for another reason.

A11. If an appellant filed an appeal brief in compliance with the requirements of former 37 CFR 1.192 (c) before September 13, 2004, the effective date, but the fee for the brief was filed on or after September 13, 2004 within the time period under former 37 CFR 1.192(a), would the appellant have to file an amended brief in compliance with 37 CFR 41.37(c)? [added 19Oct2004]

No, the Office will accept an appeal brief filed before September 13, 2004, that complies with either former 37 CFR 1.192(c) or new 37 CFR 41.37(c), even if the fee for the brief was timely filed on or after September 13, 2004.

A12. Appellant filed an appeal brief prior to September 13, 2004, the effective date of the BPAI final rule. The examiner reopened the prosecution and issued a non-final Office action with a new ground of rejection prior to September 13, 2004. Can the appellant reinstate the appeal by filing a reply brief that addresses the new ground of rejection? [added 4Jan2005]

Yes, appellant may reinstate the appeal by filing a reply brief that addresses the new ground of rejection. The appeal can also be reinstated by filing a complete new brief in compliance with 37 CFR 41.37. If instead of the Office action being mailed prior to September 13, 2004, the Office action was mailed on or after September 13, 2004, appellant cannot reinstate the appeal by filing a reply brief that addresses the new ground of rejection but instead can only be reinstated by filing a complete new brief in compliance with 37 CFR 41.37.

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B. Time Periods for Filing Appeal Brief, Response to Examiner's Answer, etc.

B1. If the appellant files a notice of appeal with a certificate of mailing under 37 CFR 1.8, which date (the date on the certificate of mailing or the date of receipt by the Office) will start the two month time period for filing the brief?

The date of receipt by the Office (or date of deposit with the USPS as set forth in 37 CFR 1.10) of the notice of appeal will be used as the date of filing the notice of appeal as set forth in 37 CFR 41.37(a). See MPEP § 512. The brief is required to be filed within two months from the date of filing the notice of appeal. The time period allowed for reply to the action from which the appeal was taken is no longer relevant. Appellant may petition for extensions of time under 37 CFR 1.136(a) for patent applications or 37 CFR 1.550(c) for reexamination proceedings.

B2. Will extensions of time be available for filing an appeal brief, a reply in response to a new ground

of rejection in an examiner's answer, and a reply brief for a patent application?

	Time period for filing	**Are extensions of time under § 1.136(a) available for patent applications**	**Are extensions of time under § 1.136(b) available for patent applications?**	**Are extensions of time under § 1.550(c) available for *ex parte* reexamination proceedings?**
Appeal brief	Two (2) months from the receipt date of a notice of appeal (see § 41.37(a)(1)).	Yes, up to five (5) months after the two month time period for filing the brief (see § 41.37(e)).	Yes (see § 41.37 (e)).	Yes (see § 41.37 (e)).
Amended appeal brief filed in response to a notice of non-compliant appeal brief	Thirty (30) days or one (1) month, whichever is longer, from the date of mailing a notice of non-compliant appeal brief (see § 41.37(d)).	Yes, up to five (5) months after the 30 days or 1 month time period for filing the amended brief (see § 41.37 (e)).	Yes (see § 41.37 (e)).	Yes (see § 41.37 (e))
A reply under §1.111 or a reply brief in response to a new ground of rejection in an examiner's answer	Two (2) months from the date of mailing of the examiner's answer (see § 41.39 (b)).	No (see § 41.39 (c)).	Yes (see § 41.39 (c)).	Yes (see § 41.39 (c))
A reply brief under §41.41 or §41.43	Two (2) months from the date of mailing of the examiner's answer (see § 41.41 (a)(1) or § 41.43(b)).	No (see § 41.41 (c) or § 41.43(c)).	Yes (see § 41.41 (c) or §41.43(c))	Yes(see § 41.41 (c) or §41.43(c))
A reply under §1.111 or a reply brief in response to a supplemental examiner's answer written in response to a remand by the Board for further consideration of a rejection under §41.50 (a)(2).	Two (2) months from the date of mailing of the supplemental examiner's answer (see § 41.50(a)(2)).	No (see § 41.50 (f)).	Yes (see § 41.50 (f)).	Yes (see § 41.50 (f)).

37 CFR 1.136(b) provides that when a reply cannot be filed within the time period set for such reply and §

1.136(a) is not available, the period for reply will be extended only for sufficient cause and for a reasonable time specified. Any request for an extension of time under § 1.136(b) must be filed on or before the day on which such reply is due, but the mere filing of such a request will not effect any extension.

37 CFR 1.550(c) provides that the time for taking any action by a patent owner in an *ex parte* reexamination proceeding will be extended only for sufficient cause and for a reasonable time specified. Any request for an extension of time must be filed on or before the day on which such reply is due, but the mere filing of such a request will not effect any extension.

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C. Amendments and Affidavits or Other Evidence

C1. What are the different standards for admitting an amendment, or affidavit or other evidence filed after a final rejection in a patent application?

	Filed after a final rejection, but before or on the date of filing a notice of appeal	**Filed after the date of filing a notice of appeal, but prior to the date of filing an appeal brief**	**Filed on or after the date of filing an appeal brief**
Amendments	**May be admitted only to:** **· Cancel claims;** **· Comply with any requirement of form expressly set forth in a previous action;** **· Present rejected claims in better form for consideration on appeal; or** **· Amend the specification or claims upon a showing of good and sufficient reasons why the amendment is necessary and was not earlier presented.** **See § 1.116(b).**	**May be admitted as provided in § 1.116 (b). See § 41.33(a) and previous column.**	**May be admitted only to:** **· Cancel claims, where such cancellation does not affect the scope of any other pending claim in the proceeding; or** **· Rewrite dependent claims into independent form.** **See § 41.33 (b).**
	May be admitted upon a showing of good and sufficient reasons why the affidavit or other evidence is necessary and was not earlier presented. See §1.116 (e).	May be admitted if it: · Overcomes all rejections under	Affidavits or other evidence may not be admitted. See §41.33 (d)(2).

Affidavits or Other evidence		appeal; and · Upon a showing of good and sufficient reasons why the affidavit or other evidence is necessary and was not earlier presented. See §41.33(d)(1).	

C2. What are some of the examples of "other evidence" under § 1.116(e) and § 41.33(d)?

The term, "other evidence," includes: declarations and exhibits, but it does not include information disclosure statements (IDS).

C3. If an amendment is filed with a notice of appeal after a second non-final Office action (and not a final rejection), which rule applies to the amendment?

37 CFR 1.111 would apply to such an amendment. 37 CFR 1.116 would not apply since it is limited to amendments and affidavits or other evidence filed after final action and prior to or with any appeal. If the amendment were filed after a notice of appeal, 37 CFR 41.33 would apply.

C4. Would the examiner need to send out an advisory action in reply to an affidavit or other evidence that is denied entry?

Yes. The examiner must notify applicant as to whether or not the paper is entered for purposes of appeal.

C5. What happens if the applicant does make a showing that there is good and sufficient reasons for entry of an affidavit or other evidence filed after a notice of appeal but prior to the appeal brief, but the affidavit or other evidence only removes one of the grounds of rejection (*e.g.*, removes the rejection under 35 U.S.C. 103(a) of claims 6-10, but not the rejection under 35 U.S.C. 102(a) of claims 1-5)?

Such an affidavit or other evidence will not be entered for purposes of appeal since it does not overcome **all** rejections under appeal. 37 CFR 41.33(d)(1).

C6. If appellant files an amendment, a notice of appeal and an appeal brief on the same date after the mailing of a final action, which rule applies to determine whether such an amendment may be admitted?

Section 1.116 applies in this situation. Such an amendment filed after a final action is not entered as a matter of right. It may be admitted if it cancels claims or complies with any requirement of form expressly set forth in a previous Office action; presents rejected claims in better form for consideration on appeal; or amends the specification, including claims, upon a showing of good and sufficient reasons why the amendment is necessary and was not earlier presented.

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D. Notice of Appeal

D1. If at least one of the claims in a patent application is rejected for the second time but not finally rejected in a non-final Office action, can applicant appeal the rejections set forth in the non-final Office action by filing a notice of appeal?

Yes, § 41.31 provides that an applicant, any of whose claims has been twice rejected, may appeal from the decision of the examiner to the Board by filing a notice of appeal accompanied by the fee set forth in § 41.20 (b)(1) within the time period for reply provided in the non-final Office action.

D2. If prosecution is reopened and a subsequent appeal is taken, will applicant be required to again file a notice of appeal and an appeal brief and pay the notice of appeal and appeal brief fees?

If appellant wishes to file a second appeal, appellant must file a second notice of appeal and an appeal brief in compliance with § 41.37(c)(1). Once the notice of appeal and appeal brief fees have been paid, however, a second set of notice of appeal and appeal brief fees will not be required except if a final Board decision has been made on the first appeal. See *Rules of Practice Before the Board of Patent Appeals and Interferences*, **69 Fed. Reg. 49959** *[PDF]*, 49975 (August 12, 2004) (final rule)), answer to Comment 44. If, however, the fees set forth in 37 CFR 41.20 have increased since they were previously paid, then applicants must pay the difference between the current fee(s) and the amount previously paid.

D3. How does an applicant reinstate an appeal? [updated 4Jan2005]

An applicant can reinstate an appeal by filing a second notice of appeal in compliance with 37 CFR 41.31 and a complete new brief in compliance with 37 CFR 41.37. Any fees paid for the notice of appeal, appeal brief, and request for an oral hearing (if applicable) will be applied to the reinstated or second appeal on the same application as long as a final Board decision has not been made on the first appeal. If, however, the appeal fees set forth in 37 CFR 41.20 have increased since they were previously paid, then applicants must pay the difference between the current fee(s) and the amount previously paid.

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E. Appeal Brief

E1. If appellant only presents arguments for a dependent claim but not for the independent claim in a group of claims that are subject to the same ground of rejection (*e.g.*, appellant only argues that the reference cited does not show one of the limitations of the dependent claim, but it is not a limitation of the independent claim), how should the examiner treat the independent claim in the examiner's answer?

The examiner should fully explain how the limitations of the independent claim are rejected and address the appellant's arguments regarding the dependent claim.

E2. Does the examiner have to check the claims presented in the claims appendix?

Yes, the examiner should check the claims presented in the claims appendix to the extent that the examiner has a reasonable assurance that the correct claims are set forth in the appendix.

E3. If appellant fails to include a copy of the claims involved in the appeal in the claims appendix section of the appeal brief or the claims in the appendix are not the correct claims, can the examiner provide an accurate copy of the claims on appeal in an appendix to the examiner's answer?

Yes, the examiner may provide a copy of the claims involved in the appeal in an appendix to the examiner's answer, or object to the appeal brief and require appellant to provide the claims appendix.

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F. Examiner's Answer (Including New Ground of Rejection (§ 41.39)

F1. If the appellant requests that the prosecution be reopened by filing a reply in compliance with 37 CFR 1.111 in response to an examiner's answer that contains a new ground of rejection, can the examiner make the next Office action final?

Yes, the examiner may make the next Office action final unless the examiner introduces a new ground of rejection that is neither necessitated by the applicant's amendment of the claims nor based on information submitted in an information disclosure statement filed during the period set forth in 37 CFR 1.97(c) with the fee set forth in 37 CFR 1.17(p). See MPEP 706.07(a).

F2. Can the Office deny a request to reopen prosecution under § 41.39(b)(1) when appellant files a reply in compliance with § 1.111 in response to a new ground of rejection in an examiner's answer?

No, if appellant files a reply in compliance with § 1.111 in response to an examiner's answer that contains a new ground of rejection, the examiner must reopen the prosecution by entering and considering the reply.

F3. How should the examiner treat a request to reopen prosecution under § 41.39(b)(1) that is accompanied by a non-responsive reply?

If the reply is not fully responsive to the new ground of rejection, but the reply is *bona fide*, examiner should provide a 30-day or 1-month time period, whichever is longer, for appellant to complete the reply pursuant to § 1.135(c). If the reply is non-*bona fide* (*e.g.*, does not address the new ground of rejection by presenting a new argument or amended claims), examiner must *sua sponte* dismiss the appeal as to the claims subject to the new ground of rejection if the two month time period to reply to the examiner's answer has expired. The examiner should notify the appellant that they have the balance of any remaining time period to correct the non-bona fide reply. Extensions of time under § 1.136(a) are not applicable. 37 CFR 41.39(c). It would be improper for the examiner to *sua sponte* dismiss the appeal until the time period for reply has expired.

F4. If an examiner indicates in the advisory action that the amendment after final rejection will be entered for purposes of appeal, and the advisory action indicates which rejection set forth in the action from which appeal has been taken would be used to reject the added or amended claims (this is the exception provided in former § 1.193(a)(2)), would such a rejection be considered a new ground of rejection when it is included in an examiner's answer?

No, such a rejection will not be considered a new ground of rejection in an examiner's answer. If such a rejection is included in an examiner's answer, the examiner will not be required to obtain the approval of the TC Director or designee, and appellant will not have the right to request that prosecution be reopened.

F5. If appellant alleges that a rejection in an examiner's answer is a new ground of rejection, but the examiner did not designate the rejection as a new ground of rejection, can the appellant file a petition under § 1.181 for the TC Director's supervisory review of the examiner's answer?

Yes, appellant may file a petition under § 1.181 within two months from the mailing of the examiner's answer (see § 1.181(f)). The petition should include reasons why the rejection should be designated as a new ground of rejection. No petition fee is required. Any allegation that an examiner's answer contains a new ground of rejection but not designated as such is waived if not timely (§ 1.181(f)) raised by way of a petition under § 1.181(a). See MPEP 1208.01.

The filing of the petition does not toll any time period running, therefore if appellant wishes to present

arguments to address the rejection in the examiner's answer, appellant must also file a reply brief to an examiner's answer within two months from the mailing date of the examiner's answer. If the TC Director or designee decides that the rejection is considered a new ground of rejection, the examiner would be required to send a corrected examiner's answer that identifies the rejection as a new ground of rejection in a separate heading in the "Grounds of Rejection to be Reviewed on Appeal" and "Grounds of Rejection" sections of the examiner's answer and include the approval of the TC Director or designee. The appellant may then file a request that the prosecution be reopened by filing a reply under § 1.111, or a request that the appeal be maintained by filing a reply brief or resubmitting the previously-filed reply brief, within two months from the mailing of the corrected examiner answer.

F6. If appellant files a request that prosecution be reopened, but fails to file a reply in compliance with § 1.111 to address the new ground of rejection in the examiner's answer, should the entire appeal be dismissed?

No, the entire appeal should not be dismissed if there are other claims on appeal that are not subject to the new ground of rejection. Section 41.39(b) provides only for dismissal of the appeal as to the claims subject to the new ground of rejection. See **F3** regarding non-responsive replies.

F7. If all of the claims under appeal are subject to the new ground of rejection provided in the examiner's answer and appellant fails to file a reply in compliance under § 1.111 or a reply brief within the time period set forth in § 41.39(b), will the entire appeal be dismissed?

Yes, the entire appeal will be dismissed if all of the claims under appeal are subject to the new ground of rejection. The examiner should follow the procedure set forth in MPEP 1215, *e.g.*, if there are no allowed claims, the application would be abandoned.

F8. If appellant fails to respond to an examiner's answer that contains a new ground of rejection, who would dismiss the appeal as to the claims subject to the new ground of rejection?

If all of the claims under appeal are subject to the new ground of rejection, the examiner should dismiss the entire appeal by following the procedure set forth in the MPEP 1215 (*e.g.*, if no claims stand allowed, the application is considered as abandoned on the date after the reply or reply brief is due).

If only some of the claims under appeal are subject to the new ground of rejection, the examiner should notify the appellant that the appeal as to the claims subject to the new ground of rejection is dismissed and the claims subject to the new ground of rejection are canceled. The dismissal of the appeal as to some of the claims on appeal operates as an authorization to cancel those claims and the appeal continues as to the remaining claims. Examiner may use the following **draft form paragraph** to notify the appellant:

> Appellant failed to respond to the examiner's answer that includes a new ground of rejection mailed on **[1]**. Under 37 CFR 41.39, if an examiner's answer contains a rejection designated as a new ground of rejection, appellant must, within two months from the date of the examiner's answer, file either: (1) a request that prosecution be reopened by filing a reply under 37 CFR 1.111; or (2) a request that the appeal be maintained by filing a reply brief under 37 CFR 41.41, to address each new ground of rejection, to avoid *sua sponte* dismissal of the appeal as to the claims subject to the new ground of rejection. In view of appellant's failure to file a reply or a reply brief within the time period set forth in 37 CFR 41.39, the appeal as to claims **[2] is dismissed, and these claims are canceled.** Only claims **[3]** remain in the application. The appeal continues as to these remaining claims. The application will be forwarded to the Board after mailing of this communication.
>
> **Examiner Note:**
> 1. In bracket 1, insert the mailing date of the examiner's answer.
> 2. In bracket 2, insert the claim numbers of the claims subject to the new ground of rejection.
> 3. In bracket 3, insert the claim numbers of the claims that are not subject to the new ground of rejection.

F9. If examiner wants to cite a judicial decision that was published in the USPQ and West Reporter System in an examiner answer, where can the examiner find both citations?

The USPQ link on the patent examiner's toolkit can be used to obtain a West Reporter System citation. Just put the case name in the search box, select "All Collections" in the box, and hit "Go." In the "All Collections Results," in the Parallel Citations (USPQ2d and USPQ) row, hit "Go" to obtain the West Reporter System citation.

F10. How does a Technology Center Director, or his or her designee, indicate approval of a new ground of rejection (or a supplemental examiner's answer)?

The Technology Center Director, or his or her designee would indicate approval of a new ground of rejection (or a supplemental examiner's answer) by stamping or writing "New Ground of Rejection Approved" on the first page of the examiner's answer (or a supplemental examiner's answer) along with the approving person's printed name, signature and the date, or by providing a similar clear indication on the examiner's answer (or a supplemental examiner's answer) that the action has been approved.

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G. Reply Brief (§ 41.41)

G1. If a reply brief is filed in response to an examiner's answer that includes a new ground of rejection, can the examiner provide a supplemental examiner's answer to respond to any new issue raised in the reply brief? Can the appellant request that prosecution be reopened after such a supplemental examiner's answer?

The examiner may provide a supplemental examiner's answer, with the Technology Center Director's or his or her designee's approval, to respond to the new issue(s) raised in the reply brief. The appellant cannot request that prosecution be reopened after such a supplemental examiner's answer. The appellant may file another reply brief under § 41.41 to any supplemental examiner's answer within 2 months from the date of the supplemental examiner's answer. 37 CFR 41.43(b). Extensions of time under § 1.136(a) are not applicable.

G2. Does the examiner have to enter a reply brief that is filed in compliance with § 41.41?

Yes, § 41.43 provides that the primary examiner must acknowledge receipt and entry of the reply brief (which is the same procedure as in the former § 1.193(b)(1)). A reply brief that is not filed in compliance with § 41.41 (a) (*e.g.*, the reply brief is not timely filed within two months from the date of the examiner's answer or it includes a new or non-entered amendment), however, will not be considered. The examiner will notify the appellant that the reply brief is non-compliant.

G3. Can appellant, in a reply brief, present arguments that are not limited to the new ground of rejection or new issues raised in the examiner's answer?

Yes, appellant may include other new issues in a reply brief. Appellant is not limited to responding only to the new rejection or new issue raised in the examiner's answer. However, new or non-admitted amendments/affidavits must not be included. 37 CFR 41.41(a)(2).

G4. What are some examples of a new issue raised in a reply brief which would provide the examiner the option to file a supplemental examiner's answer?

Example 1: The rejection under 35 USC 103 over A in view of B. The brief argues that element 4 of reference B cannot be combined with reference A as it would destroy the function performed by reference A. The reply brief argues that B is nonanalogous and therefore the two references cannot be combined.

Example 2: Same rejection as Example 1. The brief argues only that the pump means of claim 1 is not taught in the applied prior art. The reply brief argues that the particular retaining means of claim 1 is not taught in the prior art.

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H. Supplemental Examiner's Answer (§41.43)

H1. When may an appellant request that prosecution be reopened pursuant to § 41.50(a)(2) in response to a supplemental examiner's answer?

Appellant may request to reopen prosecution only if (1) a supplemental examiner's answer is written in response to a remand by the Board; (2) the remand is for further consideration of a rejection; and (3) the remand is on or after the effective date. If the Board does not remand the application to the examiner or the remand is for another reason (*e.g.*, for consideration of a new issue raised in a reply brief), the appellant cannot request that prosecution be reopened under § 41.50(a)(2) in response to a supplemental examiner's answer.

H2. If the examiner writes a supplemental examiner's answer responding to a new issue raised in a reply brief, and then the appellant files a second reply brief that raises another new issue, can the examiner provide a second supplemental examiner's answer to respond to the new issue raised in the second reply brief?

Yes, with the Technology Center Director's, or his or her designee's, approval.

H3. Do all remands from the Board in which the examiner introduces a supplemental examiner's answer provide appellant the opportunity to have the prosecution reopened under 37 CFR 41.50?

No. Only remands which are **in further consideration of a rejection** shall provide appellant the right to reopen prosecution under 37 CFR 41.50. An example of a remand in further consideration of a rejection is where the Board has knowledge of a ground of rejection not involved in the appeal and remands the application for consideration of such ground of rejection. Certain remands from the Board will not give the appellant the right to reopen prosecution (*e.g.*, administrative remands, a remand to consider an IDS submitted after the jurisdiction has shifted to the Board and a remand to consider a reply brief not addressed by the examiner).

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I. Other Topics

I1. Is the revised *ex parte* appeal practice provided in the current MPEP?

The current MPEP (Eighth Edition, Second Revision, May 2004) does not include the revised appeal practice. . The revised appeal practice will be incorporated into the next revision of the MPEP.

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Last Modified: 01/06/2005 14:42:50

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

BOARD OF PATENT APPEALS AND INTERFERENCES

Attorney Docket No. 071007/0137

In re application of:

Group Art Unit: 1641

Serial No.: 08/886,044

Examiner: S. Devi

Filing Date: June 30, 1997

For: VACCINE AGAINST GRAM-NEGATIVE BACTERIAL INFECTIONS

ADDITIONAL EVIDENCE SUBMITTED MPEP §1207

Commissioner of Patents
Customer Service Window
Randolph Building
401 Dulany Street
Alexandria, VA 22314

Sir:

This communication is responsive to a telephonic request by Examiner Devi, received on February 15, 2005, that concerns the captioned application, presently on appeal.

According to the examiner, certain articles were provided for the first time with the original version of Appellant's Brief (see pages 11 and 13 of the "Fourth Revised Brief on Appeal," filed concurrently) and, hence, must be submitted via a separate paper, pursuant to Section 1207 of the MPEP. She identifies these articles as (i) Zanetti *et al.*, "Use of Immunoglobulins in Prevention and Treatment of Infection in Critically Ill Patents: Review and Critique," *Reviews of Infectious Diseases* 13: 985-92 (1991), and (ii) Glauser *et al.*, "Pathogenesis and Potential Strategies for Prevention and Treatment of Septic Shock: Un Update," *Clinical Infectious Diseases* 18 (Suppl 2):S205-16 (1994). A copy of each article accompanies the present paper.

Zanetti *et al.* (1991) is relevant to the pending appeal for its statements that "protection was related to immune plasma, not to specific levels of antibody to core LPS in a given plasma" (page 988, first paragraph) and that, "in both successful clinical studies with *E.*

coli J5 antiserum, the protection remained of unclear origin because outcome could not be convincingly correlated with the level of antibodies to the core LPS of *E. coli* J5...the protection afforded by *E. coli* J5 antiserum could not be attributable to antibodies to the LPS of *E. coli* J5" (*id.*, second full paragraph). Similarly relevant is the conclusion in Glauser *et al.* (1994) that "a favorable outcome could not be correlated with antibody titers in either of the two clinical studies done with human polyclonal antisera to J5...the mechanisms of protection by antisera to J5 remain unknown" (second full paragraph on page S208).

As recently as 1997, therefore, both Zanetti and Glauser had characterized as failures the subject clinical trials, where the use of an anti-J5 LBS monoclonal antibody fared no better, in providing protection, than did polyclonal antiserum.

Appellants respectfully request entry of Zantetti *et al.* and Glauser *et al.* into the record for the appeal.

Respectfully submitted,

18 February 2005
Date

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Use of Immunoglobulins in Prevention and Treatment of Infection in Critically Ill Patients: Review and Critique

Giorgio Zanetti, Michel P. Glauser, and Jean-Daniel Baumgartner

From the Division of Infectious Diseases, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland

The study of the use of standard intravenous immunoglobulin (IVIG) preparations as adjunctive therapy for seriously ill patients is motivated by the need to restore immunoglobulin G depleted because of trauma or surgery and/or by the need to provide patients with specific antibodies to various microorganisms. Whereas no clinical studies have shown that standard IVIG has therapeutic efficacy, some data suggest that its prophylactic use is beneficial. Antisera or IVIG prepared from individuals who are hyperimmunized with the biologically active, highly conserved core portion of the endotoxin of gram-negative bacteria confer variable degrees of protection in animal models and clinical trials. Two clinical trials with use of monoclonal antibodies to core lipopolysaccharide have been completed. Only subsets of patients with gram-negative sepsis were protected by the monoclonal antibodies, but the results of the studies were discrepant in regard to the specific characteristics of patients who benefited from the administration of these antibodies. Further studies will be necessary to establish whether this therapy can be recommended for critically ill patients.

Critically ill patients are at high risk for infection as a result of several immunologic dysfunctions. Decreased levels of IgG have been reported in such patients, especially after trauma and surgery [1]. Moreover, the efficacy of their neutrophils undergoes a decline with respect to exhibition of chemotaxis, opsonic activity, and ability to kill bacteria. In addition, such patients are exposed to a wide variety of organisms in the hospital environment, a circumstance which further increases the probability of infection. The administration of intravenous immunoglobulin (IVIG) to critically ill patients might enhance host defense by restoring IgG and/or by providing patients with specific antibodies to various microorganisms or constituents of microorganisms. IVIG might also attenuate the inflammatory process engendered by the host response to bacterial products such as endotoxin. Two approaches have been used in the administration of IVIG for treatment of critically ill patients: (1) administration of standard IVIG (nonspecific use) and (2) administration of whole plasma, immunoglobulin preparations, or monoclonal antibodies (MoAbs) directed against the endotoxin constituent of gram-negative bacteria (specific use).

This article is part of a series of papers in the present issue of *Reviews* on the evolving use of biologicals in the treatment and prevention of infectious diseases. The papers were presented at a symposium held during the Interscience Conference on Antimicrobial Agents and Chemotherapy in October 1990 in Atlanta. The symposium was supported by an educational grant from Amgen Inc., Thousand Oaks, California.

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Reviews of Infectious Diseases 1991;13:985–92

0162-0886/91/1305-0048$02.00

Nonspecific Use of IVIG

Treatment of infections. IVIG can be administered to patients therapeutically for infections. In an unblinded, randomized, controlled clinical trial with 104 surgical intensive care patients, Just and colleagues [2] administered four 100-mL doses of IVIG (Pentaglobin; Biotest Pharma, Frankfurt, Germany) over 2 days in conjunction with antibiotics to 50 patients at the first sign of infection. Fifty-four control patients received antibiotics alone. The mortality attributed to infections and the overall mortality of the IVIG and control groups did not differ. The IVIG reportedly was effective for patients preoperatively classified as "high risk," but since this result was obtained in only one of several subgroups, the data should be interpreted cautiously. In a multicenter, unblinded, randomized, controlled clinical trial among 288 patients with fibrinopurulent peritonitis, Jesdinsky and co-workers [3] administered 10 g of an IVIG preparation (immunoglobulin 7S human iv; Armour Pharmaceuticals, Eschwege, Germany) to 145 patients; a control group that consisted of 143 patients was not treated. The study failed to demonstrate the efficacy of IVIG therapy, however. One possible explanation for such a result is that the amount of specific antibodies might have been insufficient to confer protection against the large number of pathogenic bacteria.

Prophylactic use of IVIG. IVIG can also be administered prophylactically. Two reports of studies using this approach have been published. Duswald and colleagues [4] administered 2.5 g of IVIG (Intraglobulin; Biotest Pharma) to 150 critically ill patients, but the investigators observed no protective effects with respect to wound infection, urinary tract infection, or pneumonia. Glinz and associates [5] administered 36 g (12 g on days 0, 5, and 12 after admission to the inten-

sive care unit) of IVIG (Sandoglobulin; Sandoz, Basel, Switzerland) to 150 patients and observed a decrease in the incidence of pneumonia. This benefit was not unequivocally proven, however, since pneumonia was mainly defined radiologically and microbiologic documentation was questionable.

Recently, Cometta and co-workers [6] completed a blinded, placebo-controlled clinical trial with two IVIG preparations. A 400 mg/kg dose of standard preparation (Gammagard; Hyland Therapeutics Division, Glendale, CA) was administered to 109 patients when they were admitted to the surgical intensive care unit and once a week thereafter. A second preparation of IVIG, which was enriched with antibodies to the core lipopolysaccharide (LPS) of *Salmonella minnesota* R595 (Re LPS) (see next section), was administered to 108 patients. The titer of the antibodies to core LPS in this preparation was eightfold higher than that in the standard IVIG. A control arm of 112 patients received albumin. The standard IVIG preparation accounted for a reduction in the number of patients who developed infections during the course of the study (36%, versus 53% in the control group; $P < .05$). The reduction was caused by a significant decline in the number of cases of pneumonia ($P < .05$).

Therefore, it appears that standard IVIG can have a protective effect when administered to high-risk surgical patients as a prophylactic measure. Indeed, detailed analysis of the results showed that both the stay in the intensive care unit and the entire duration of hospitalization were shorter for those patients who received standard IVIG than for those who received albumin. It is surprising that the IVIG enriched with antibodies to the endotoxin core failed to protect the patients from infection. The preparation conferred no protection against gram-negative sepsis, septic shock, or focal infections; the reason for this is unclear.

Antibodies to Gram-Negative Bacteria

It is well known that the toxicity of gram-negative bacteria is caused by the toxic LPS, called endotoxin, that constitutes the outer bacterial membrane. The endotoxin molecules consist of the toxic moiety, a lipoidal acylated glucosamine disaccharide (lipid A), which is linked to a polysaccharide side chain (called O antigen) through an intermediate oligosaccharide region, the core. Antibodies to the intact LPS are produced mainly in opposition to the side chains, which are highly antigenic. Because side chains vary widely between strains, these antibodies have a very narrow specificity. By contrast, the core is highly conserved and is very similar in different strains.

In the 1970s, Braude et al. [7], Ziegler et al. [8], and McCabe [9] all hypothesized that by stimulating production of antibodies to the highly conserved core moiety of LPS, one could obtain cross-reacting antibodies that would protect against a wide variety of gram-negative bacteria. This was ultimately accomplished by using the *Escherichia coli* mutant J5, a rough mutant of *E. coli* 0111:B4 that lacks the enzyme uridine 5'-diphosphonate-galactose 4-epimerase; this defect prevents attachment of the side chains to the core. Therefore, in the J5 strain, the polysaccharide side chains of the endotoxin are missing and the core is exposed. In animals, immunization with LPS from *E. coli* J5 or with whole *E. coli* J5 bacterial cells results in high titers of antibody to epitopes of the LPS core [10]. Another rough mutant that has been studied for its ability to stimulate production of antibodies to the core region is an Re mutant of *S. minnesota*.

Researchers have postulated that antibodies elicited by these two rough mutants should afford protection against a wide range of gram-negative bacteria by a mechanism requiring two steps: (1) these antibodies recognize epitopes of the core region shared by the LPS of pathogenic gram-negative bacteria, and (2) the postulated binding neutralizes the endotoxic properties of LPS, i.e., leads to a suppression or attenuation of the release of mediators such as cytokines. However, neither of these two hypothetical steps has been unequivocally demonstrated. The following sections review what has been learned from studies in which the J5 mutant and other rough mutants were used.

Studies with animals. Ziegler and colleagues have shown that granulocytopenic rabbits challenged in the conjunctival sac with *Pseudomonas aeruginosa* develop a massive, lethal pseudomonal infection [11] and that these rabbits could be protected with *E. coli* J5 antiserum. Some of their results with use of purified immunoglobulins from human volunteers immunized with *E. coli* J5 are reproduced in table 1 [12]. Braude et al. [7], McCabe [9], and other researchers [8, 13–18] have also found that animals were protected when they were actively or passively immunized with the rough mutant J5 of *E. coli* or with an Re rough mutant of *S. minnesota*.

Some investigators, however, were unable to obtain similar results [19–28]. For instance, Greisman and Johnston [27] found that mice inoculated with LPS from *S. minnesota*, *Salmonella typhimurium*, *E. coli* 0127, or *E. coli* 0111 were not protected by either of the two antisera to the J5 and Re mutants; the mortality among these mice was even greater than the mortality for mice that received only saline. The study revealed that only antibody to the strain-specific LPS was protective (table 2).

Thus, the results of animal studies described in this section

Table 1. Level of protection of neutropenic rabbits from lethal pseudomonal bacteremia after intravenous administration of immunoglobulin from humans immunized with *E. coli* J5.

Source of immunoglobulin	No. of deaths/no. of rabbits treated (%)*
Nonimmunized human	12/14 (86)
Immunized human	3/14 (21)

NOTE. Reprinted with permission from [12].
* $P = .001$ (χ^2 test).

Table 2. Level of protection (in terms of mortality) noted in mice after pretreatment with various preparations* and inoculation with 230 μg of LPS from *S. typhimurium*.

Pretreatment preparation (0.5 mL)	Percentage of deaths/ no. of mice inoculated
Saline	52/25
Antiserum to *E. coli* J5†	75/12
Preimmune serum†	64/11
Antiserum to *E. coli* J5‡	83/12
Preimmune serum‡	83/12
Antiserum to *S. typhimurium*	0/25§

NOTE. Adapted with permission from [27].
* The *E. coli* J5 antisera were prepared by using the method of Braude et al. [7].
† Serum from rabbit 25.
‡ Serum from rabbit 26.
§ $P < .0005$, compared with the other trials.

Table 3. Mortality among patients with gram-negative bacteremia after administration of control serum from nonimmunized donors or antiserum from donors immunized with *E. coli* J5.

Patient group	No. of deaths/no. of patients treated (%) in group receiving indicated therapeutic serum: Control serum (mean titer, 1:6)	Antiserum (mean titer, 1:32)	*P* value
All patients	38/100 (38)	22/91 (24)	.04
Patients in profound shock	26/34 (76)	17/37 (46)	.009

NOTE. Adapted with permission from [36].

show that only some researchers have been successful in demonstrating that antibodies to core glycolipids can be protective. There is no simple explanation for the discrepant results of studies with various preparations of antibodies to core LPS. The precise specificities as well as the mode of action of the antibodies tested have not been clarified. Moreover, the animal models studied, the mode of challenge, and the nature of LPS or of bacteria used for challenge are parameters that all could have an impact on the protective efficacy of these preparations [29].

However, these considerations cannot explain all the discrepancies in data; varying results were sometimes obtained despite the use of similar antibodies, similar animal models, and similar bacterial or LPS challenges [8, 9, 26, 27, 30]. Therefore, it can be postulated that additional, unknown negative or positive factors sometimes operate. For instance, an artifact (now well recognized) can result when the antibody preparation to be tested is contaminated by LPS. Minute amounts of LPS administered prophylactically induce a state of tolerance to LPS and protect animals against subsequent bacterial or LPS challenges [31, 32]. Since antibodies tested in studies of protection almost uniformly are administered before bacterial or LPS challenge, the occurrence of such an artifact in many earlier-reported experiments cannot be ruled out. Moreover, the interpretation of data from experimental studies of protection with use of rabbit antisera may depend on whether preimmune and immune sera came from the same rabbit or from different rabbits [27]; it has been shown that sera from nonimmunized rabbits, unlike other sera, may have a natural protective power against challenge with gram-negative bacteria or LPS.

Similarly, in studies of protection with use of MoAbs, the degree of purification is critical to the results. Indeed, ascitic and hybridomal fluids can contain various proteins and peptides, such as cytokines, some of which might be able to bind to LPS or to induce some tolerance to LPS in experimental animals. Most experimental studies of protection that have been reported, including those by Teng et al. [30] and Young et al. [33], have involved ascitic or hybridomal fluids. Experimental variability might account for important differences in survival rates noted from one experiment to another, so the reporting of only part of the experiments might introduce some element of bias [34, 35]. In conclusion, the precise description of the control preparation and of the preparation containing antibody is critical for the interpretation of experimental data from studies with animals.

Clinical studies. As was the case with the animal studies described in the previous section, only some of the data from clinical studies indicated that patients could be successfully treated or protected with administered antibodies.

In 1982 Ziegler and co-workers [36] reported a pioneering randomized, blind, controlled study of patients with gram-negative bacteremia who received either serum from healthy volunteers who had been vaccinated with heat-killed *E. coli* J5 or control serum obtained from the same donors before immunization. There was a fivefold difference in the mean titers of antibody to the LPS of *E. coli* J5 in the control and immune serum. These sera were administered to 304 patients, 191 of whom had gram-negative bacteremia. The results (table 3) led to the conclusion that human *E. coli* J5 antiserum substantially reduces mortality due to gram-negative bacteremia and septic shock. Regarding the mode of protection, Ziegler et al. [36] hypothesized that antibodies to *E. coli* J5, which were present in the serum after immunization, became bound to some part of the endotoxin core of pathogenic gram-negative bacteria and sterically prevented lipid A from reacting with mediators of shock in blood and tissue fluid. This hypothesis could not be convincingly confirmed, however, since the outcome could not be significantly correlated with the level of antibody to *E. coli* J5 measured in the serum administered to patients.

In a randomized, double-blind, prophylactic trial, Baumgartner and colleagues [37] were able to show that plasma from volunteers immunized with *E. coli* J5 protected surgical patients who were at high risk for gram-negative infection from shock and death. On admission to the intensive care

Table 4. Prophylaxis of surgical patients for gram-negative shock with plasma from nonimmunized donors or from donors immunized with *E. coli* J5.

Patient group	No. of patients treated with plasma from indicated type of donor		*P* value
	Non-immunized	Immunized	
All patients	136	126	. . .
with focal GN infections	55	45	NS
who developed GN shock	15	6	.049
who died of GN shock	9	2	.033
Patients who underwent abdominal surgery	83	71	. . .
who developed GN shock	13	2	.006
who died of GN shock	9	1	.017

NOTE. GN = gram-negative; NS = not significant. Adapted with permission from [37].

unit, patients received plasma harvested from donors who had been immunized against *E. coli* J5; the control group received plasma taken from these same donors before immunization. Six of 126 patients (4.8%) who received plasma from immunized donors and 15 of 136 patients (11.0%) in the control group developed gram-negative shock; the numbers of related deaths in each group were two (1.6%) and nine (6.6%), respectively ($P < .05$, one-tailed Fisher exact test). The difference between the two groups was observed mainly in patients who underwent abdominal surgery (table 4). Although administration of plasma with antibodies to *E. coli* J5 failed to decrease the incidence of gram-negative infection, it greatly reduced the most serious consequences of such infections. As was noted in the report by Ziegler et al. [36], protection was related to immune plasma, not to specific levels of antibody to core LPS in a given plasma (D. Heumann and J-D Baumgartner, unpublished data).

Two other studies, however, have failed to demonstrate that any beneficial effect results from the administration of *E. coli* J5 antiserum. McCutchan and colleagues [38] studied neutropenic patients as well as patients receiving bone marrow transplants. These patients were given *E. coli* J5 antiserum as a prophylactic measure. The results did not suggest that the antiserum prevented gram-negative bacteremia or the occurrence of fever that, for at least some of these patients, was considered to be caused by release of endotoxin from the gut. One possible explanation for this failure to demonstrate such a beneficial effect could be the low power of the study, since the number of infections due to gram-negative bacteria was small. Recently, we conducted a blind study in which 73 children with purpura fulminans received either control plasma (33 patients) or plasma with antibodies to the core LPS of *E. coli* J5 (40 patients) (E. Girardin and J-D Baumgartner, submitted for publication). There was no difference in mortality between the two groups, a fact suggesting that the plasma from donors immunized with *E. coli* J5 was not effective in the treatment of meningococcal septicemia.

As already noted, in both successful clinical studies with *E. coli* J5 antiserum [36, 37], the protection remained of unclear origin because outcome could not be convincingly correlated with the level of antibodies to the core LPS of *E. coli* J5 ([36] and D. Heumann and J-D Baumgartner, unpublished data). In addition, it was found that in 70 volunteers who donated their plasma for one of these studies [37], immunization with *E. coli* J5 vaccine (provided by E. J. Ziegler) induced a modest threefold increase in antibodies to the LPS of *E. coli* J5 but no increase in antibodies to Re LPS or to lipid A [39]. Thus, the protection afforded by *E. coli* J5 antiserum could not be attributable to antibodies to the LPS of *E. coli* J5, to Re LPS, or to lipid A.

IVIG enriched with antibodies to the LPS core has been purified from serum of immunized volunteers or from serum of donors with naturally acquired high levels of antibodies to LPS. Calandra and colleagues [40], in a randomized, double-blind trial, compared the efficacy of IVIG collected from volunteers who were immunized with *E. coli* J5 with that of standard IVIG (Sandoglobulin) in the treatment of 71 patients with gram-negative septic shock. There was a 2.2-fold increase in titer of antibody to the LPS of *E. coli* J5 in the preparation from the hyperimmunized donors compared with that in the standard IVIG. No difference between the two groups in terms of mortality was reported.

As previously noted, Cometta and co-workers [6] compared IVIG that contained antibodies to core LPS, which was collected from blood donors with naturally high levels of Re antibodies, with standard IVIG or placebo in a prophylactic double-blind study. In contrast to the efficacy of standard IVIG, no protection was afforded by the IVIG with core LPS antibodies. One hypothetical explanation for the ineffectiveness of IVIG with antibodies to core LPS is that IgM antibodies, which were absent from IVIG preparations, might be necessary for protection [15]. However, since the precise specificity and the mode of action of antibodies to core LPS are unknown, there is no strong basis for such a claim. Although some experimental data suggested that IgM-enriched serum fractions were more effective than IgG-enriched fractions [15], other studies had found that IgG antibodies were as effective or even more effective than IgM [17, 41].

This review of the clinical trials performed with antisera or polyclonal immunoglobulin reveals that, among six studies, four were unsuccessful in demonstrating the efficacy of the administered preparations. The data emphasize the need for an understanding of which factors were responsible for the protective effects noted in the successful studies with serum or plasma and, in addition, which of these factors were absent from the unsuccessful studies with plasma or immunoglobulin preparations.

Studies With Monoclonal Antibodies

In recent years, several MoAbs have been developed that recognize various epitopes of the core region of endotoxin [30, 33, 42–52]. Two of these MoAbs, both of the IgM class, have been tested for treatment of patients with gram-negative infections.

First, Young and associates [33] made murine MoAbs to the endotoxin core of the J5 mutant of *E. coli* and of the Re mutant of *S. minnesota*. They immunized BALB/c mice against these bacterial strains and could produce IgM MoAbs to Re LPS or lipid A. The ability of these MoAbs to prevent or treat infection was then tested in female mice. The authors found that these antibodies were not protective when administered alone [33], but one of them, an IgM MoAb to lipid A called E5, appeared to be synergistic with antibiotics in experimental studies of prophylactic or therapeutic methods.

For instance, mice were injected with a dose of live serum-resistant bacteria (three challenge organisms were used) that would be expected to kill 80%–100% of the animals. Two hours after onset of infection, a mixture of antibiotics was injected intramuscularly; control mice received saline. After an additional 2 hours, the MoAb E5 was injected intravenously.

There were four treatment groups, with 14 mice per group and per challenge organism. Group A received antibiotics alone; 45% survived (results of challenges with the three organisms were pooled). Group B received antibiotics and MoAbs; 64% survived ($P < 0.05$, one-tailed, in comparison with group A when the results of the three bacterial challenges were combined). For group C (which received MoAbs and saline) 29% survival was noted, and for group D (which received saline alone) 24% survival was noted.

In another experiment, the MoAb E5 was used for the treatment of infections due to two strains of *P. aeruginosa* in mice. Again, E5 alone had no protective effect, but when the results for the two strains were combined, treatment with E5 and antibiotics was significantly more effective than treatment with antibiotics alone. These studies suggested, therefore, that E5 might have a protective effect in some experimental conditions. However, definitive conclusions are difficult to draw because ascitic fluid, not purified antibody, was used and because individual experiments had to be pooled to obtain statistically significant differences.

In a clinical study of the MoAb E5 [53], patients with a suspected gram-negative septic syndrome were randomly assigned to receive intravenously either the antibody (2 mg/kg daily for 2 consecutive days) or an identical volume of saline. Of the 468 evaluable patients, 316 had a documented gram-negative infection. No decrease in mortality was observed in this group of patients. However, when the results in subgroups were analyzed, there was a statistically significant decrease in mortality among the 137 patients who were not in shock when enrolled in the study ($P = .03$), whereas the 179 patients who were in shock were not protected. Shock was defined as refractory hypotension; patients with organ failure or disseminated intravascular coagulation were not considered to be in shock if they had a systolic pressure >90 mm Hg. Among patients who were not in shock, a comparable reduction in mortality occurred in the bacteremic group as well as the nonbacteremic group. Administration of E5 was safe in that <2% of patients developed allergic adverse effects. Because the results of this study suggested that E5 was effective only in a subgroup of patients who were not in shock (an unanticipated finding), a confirmatory multicenter study has been initiated.

The second MoAb, subsequently designated as HA-1A, was produced by Teng and co-workers [30] from a hybridoma obtained by fusing B lymphocytes from human spleen with heteromyeloma cells. The researchers used splenocytes taken from one patient with Hodgkin's disease who was undergoing splenectomy and who had previously been vaccinated with the J5 mutant of *E. coli*. They reported that the MoAb reacted in vitro with many unrelated species of gram-negative bacteria. Moreover, the MoAb in hybridomal fluid was shown to be protective against endotoxin in the dermal Shwartzmann reaction in rabbits and against gram-negative bacteremia in mice. Protection appeared to be specific for gram-negative bacteria because the MoAb to *E. coli* J5 failed to protect against the pneumococcus, a gram-positive organism that lacks endotoxin. The results of ELISAs and binding inhibition experiments led to the conclusion that the MoAb specifically recognized lipid A [54].

These experimental observations could not be reproduced, however. Indeed, using purified MoAb instead of crude hybridomal fluid, we could not demonstrate that HA-1A could be protective [26] in models very similar to those used by Teng et al. [30]. In addition, in contrast to type-specific LPS antibodies, HA-1A did not suppress LPS-induced secretion of tumor necrosis factor in mice, a circumstance suggesting that HA-1A was not able to prevent LPS from reaching its target on macrophages [26].

Moreover, it was found that purified HA-1A bound moderately to lipid A and Re LPS but poorly to LPS from pathogenic, smooth, gram-negative bacteria. It bound to a large range of gram-negative bacteria and also to gram-positive bacteria, fungi, and lipids unrelated to lipid A, including cardiolipin and lipoproteins (use of such controls has not been previously reported [30, 54]). This broad binding pattern suggested nonspecific interactions with hydrophobic constituents and may bring into question the specificity of HA-1A for lipid A (D. Heumann and J-D Baumgartner, unpublished data).

Ziegler and colleagues [54] administered HA-1A (or albumin as a control preparation) to patients with a presumptive diagnosis of gram-negative sepsis. The patients were randomized to receive a single dose of HA-1A (100 mg intravenously) or a similar volume of human albumin. Of the 543

patients, 317 had microbiologically documented gram-negative infections; for 200 of the 317 patients, blood cultures were positive at the time of randomization. HA-1A did not reduce the mortality in the overall study population or among the 117 patients with nonbacteremic gram-negative infections. However, there was a significant decrease in mortality among the subgroup of patients with gram-negative bacteremia ($P = .014$); the decrease in mortality was most obvious among the 101 patients who were in shock when enrolled in the study.

Detailed analysis of these data indicated that, by chance, differences in risk factors between placebo and HA-1A recipients might have been present in the subgroup of 200 patients with gram-negative bacteremia at the time of randomization. Indeed, a total of 101 serious complications (e.g., disseminated intravascular coagulation, adult respiratory distress syndrome, acute hepatic failure, and acute renal failure) [54] were noted at entry in the 95 placebo recipients (mean, 1.06 per patient); 85 such complications were noted in the 105 HA-1A recipients (mean, 0.81 per patient) ($P = .07$ by comparison of Poisson distributions). The 16 additional serious complications in the placebo group might partially account for the higher mortality (13 more deaths) in this group.

Definitive conclusions for the use of MoAbs to endotoxin core are difficult to draw at present. Indeed, when the HA-1A MoAb was tested experimentally by two independent groups, it showed divergent efficacy that was possibly related to its degree of purification [26, 30]. The other antibody, the E5 MoAb, only once has been reported to be moderately efficacious in animals when tested as ascitic fluid. Moreover, the clinical results of the studies have been somewhat conflicting. For instance, in one study, the MoAbs protected predominantly patients in shock; in another study, only those patients who were not in shock were protected. In one study, only patients for whom blood cultures were positive were protected by the treatment, while in another study the protection occurred independently of the blood culture status. Thus, further studies are needed to define prospectively and specifically the types of patients who might benefit most from this therapeutic approach.

Conclusions

According to available study reports, the use of standard IVIG for the treatment of infection in critically ill patients seems ineffective. In contrast, two studies have shown a reduction of infections, mainly pneumonia, when standard IVIG preparations were administered prophylactically to chosen groups of postsurgical or trauma patients. However, no impact on mortality was demonstrated. Cost-effectiveness studies are therefore warranted.

At present, the treatment of the gram-negative septic syndrome with antibodies to lipid A or other epitopes of the core LPS should still be considered investigational. None of the preparations used in clinical studies has yet emerged as an established therapeutic modality that can be administered routinely to patients with septic shock. In the two studies investigating MoAbs, only subsets of patients with the gram-negative sepsis syndrome were protected, but both studies gave discrepant results concerning the specific characteristics of patients who were reported to benefit from the administration of these antibodies. In addition, the epitope specificity and the mode of action of the MoAbs investigated so far are still unknown. These concerns are not trivial. The indiscriminate use of such treatment might have considerable financial impact: the potential market for such antibodies has been estimated to be worth several billion dollars per year in the western countries.

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Pathogenesis and Potential Strategies for Prevention and Treatment of Septic Shock: An Update

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Septic shock is mediated by complex interactions of cells, cytokines, and humoral pathways. Clinical therapeutic strategies aimed at inhibiting selected pathways have been efficacious in subsets of patients. Experimental studies focusing on the activities of single cytokines have contributed to the understanding of the complex pathophysiology of septic shock. More precise delineation of the roles of each mechanism contributing to pathogenesis will permit the identification of subsets of patients who might benefit from particular therapeutic strategies and will guide the development of additional approaches to prevention and treatment.

Septic shock is a clinical syndrome that has become increasingly important in the last 40 years. The condition is most common among hospitalized patients, particularly those with underlying diseases. Although patients with diseases caused by "classic" gram-negative pathogens (such as plague or typhoid fever) may present with the clinical picture of septic shock, it is only since the 1950s—with the increasing incidence of disease caused by gram-negative bacilli of the normal host flora—that the sepsis/septic shock syndromes have been defined. We believe that these definitions (table 1) [1, 2] are satisfactory: the identical incidences of shock and death in the various clinical studies using these criteria indicate that similar groups of patients are being enrolled. However, the definitions need to be improved to take into account various factors that are important for predicting outcome, including underlying diseases and appropriateness of antibiotic and medical/surgical treatment. A modified scheme for classification of these syndromes—known as systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS) [3]—is currently being discussed, but no consensus has yet been reached.

Septic shock has traditionally been recognized as a consequence of gram-negative bacterial infection, but it may also be caused by gram-positive organisms and fungi and probably by viruses and parasites as well. Table 2 summarizes the organisms isolated and the mortality documented in three recent studies of sepsis syndrome/septic shock [2, 4, 5]. Gram-negative bacteria were isolated in 30%–80% of cases and gram-positive bacteria in 5%–24%. In one prospective study of sepsis syndrome [2], no etiologic agent was identified in more than half of all cases. Notably, the severity of septic shock, as reflected by mortality, did not depend on the type of organism responsible.

Initial studies of the pathophysiological features of septic shock concentrated on the interactions of lipopolysaccharide (LPS) from the gram-negative bacterial cell wall with various humoral pathways. However, attention is now focused on the central role of macrophages, endothelium, and cytokines that are released upon stimulation by most if not all of the recognized agents of septic shock (figure 1). In this review, we address the known humoral pathways that are activated during septic shock, and we discuss the role of cytokines, particularly tumor necrosis factor (TNF) and interleukin 1 (IL-1). Although the current emphasis is on the activation of macrophages and cytokine production, we think that evidence for direct activation of humoral pathways by microbial constituents remains relevant. We also review the mechanisms by which LPS interacts with macrophages, citing experimental and clinical studies evaluating the potential of anti-LPS and anticytokine agents in therapy for septic shock.

Bacterial Cell-Wall Components and Septic Shock

The exotoxins produced by some bacteria (e.g., exotoxin A produced by *Pseudomonas aeruginosa* or toxic shock syndrome toxin produced by some strains of *Staphylococcus aureus*) can initiate septic shock. However, the bacteria themselves—and in particular their cell wall components—are primarily responsible for the development of septic shock. These components are potent activators of numerous humoral pathways and also of macrophages and other cells involved in inflammatory processes.

The prime initiator of gram-negative bacterial septic shock is endotoxin, an LPS component of the bacterial outer mem-

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Table 1. Definitions of sepsis syndrome and septic shock.

Sepsis syndrome	Septic shock
Clinical evidence of infection	Sepsis syndrome with hypotension**
Tachypnea*	
Tachycardia†	
Hyperthermia or hypothermia‡	
Evidence of inadequate organ perfusion, including one or more of the following:	
Hypoxemia§	
Elevated plasma lactate concentration‖	
Oliguria#	

* Respirations, >20/min; if mechanically ventilated, >10 L/min.
† Pulse, >90/min.
‡ Core or rectal temperature, >38.3°C or <35.6°C.
§ PaO_2/FIO_2, <280 (without other pulmonary or cardiovascular disease as the cause).
‖ Exceeding upper limits of normal for the laboratory.
Documented urine output, <0.5 mL/kg of body weight for at least 1 hour (in patients with catheters).
** Sustained decrease in systolic blood pressure to <90 mm Hg or drop by >40 mm Hg for at least 1 hour when volume replacement is adequate, the patient is taking no antihypertensive medication, and other causes of shock (such as hypovolemia, myocardial infarction, and pulmonary embolism) are absent.

brane. Endotoxin circulating in the blood appears to be a predictor of poor outcome in some clinical settings (e.g., meningococcemia [6]), but the levels of endotoxin required to trigger the cascade of events in septic shock may vary greatly. Indeed, it has been observed that bacterial products other than LPS may profoundly increase the host's sensitivity to endotoxin, thereby rendering toxic otherwise-harmless levels [7]. Hence, the measurement of endotoxin has not yet become standard clinical practice.

The outermost part of the endotoxin molecule consists of a series of structurally and antigenically diverse oligosaccharides that are responsible for the O serotype of gram-negative bacteria. Internal to the O side chains are the core oligosaccharides, which have similar structures in common gram-negative bacteria. Lipid A, which is bound to the core oligosaccharide, has a highly conserved structure and is responsible for most of the toxicity of endotoxin. However, some types of natural lipid A and synthetic lipid A analogues that have different sugar and acyl residues are less—or not at all—toxic both in vitro and in vivo. This observation has led to the development of lipid A analogues that can block the toxic effects of endotoxin or act as endotoxin antagonists [8, 9].

Antibodies to Endotoxin

The O-specific oligosaccharide side chains of endotoxin are highly immunogenic. Antibodies to these side chains inhibit the effects of endotoxin and, by virtue of their opsonophagocytic properties, eradicate the endotoxin-producing organism. However, because these antibodies are specific for a particular O serotype, their clinical application is limited. An alternative approach has been to develop antibodies to the structurally conserved core glycolipid of endotoxin or to lipid A in the hope that these antibodies will offer cross-reactivity or cross-protection against the toxic component of all gram-negative bacteria. In initial clinical trials conducted 10 years ago, antisera or preparations of hyperimmune polyclonal intravenous immunoglobulin were used [10–13]. While the results of these trials suggested a benefit and stimulated subsequent trials of monoclonal antibodies, they did not specifically demonstrate that antibodies to lipid A were responsible for the protection observed.

Recently, clinical trials of two monoclonal antibodies to the core glycolipid of endotoxin have received considerable attention [14, 15]. In a prospective, randomized, placebo-controlled trial of E5 (a murine monoclonal IgM antibody to lipid A), 486 patients with suspected gram-negative sepsis

Table 2. Frequency of isolation of various types of microorganisms and corresponding mortality in three recent studies of sepsis syndrome/septic shock.

	Frequency of isolation/mortality among patients with indicated condition*		
Type of microorganism	Sepsis syndrome† (n = 382)	Septic shock‡ (n = 119)	Septic shock§ (n = 89)
Gram-negative bacteria	30/31	60/49	80/55
Gram-positive bacteria	16/29	24/89	5/2
Mixed or fungi	3/5	16/75	4/2
Not documented	53/30	...	10/11
Total	100/30	100/63	100/47

* Figures are percentages.
† Data are from [2].
‡ Data are from [4].
§ Data are from [5].

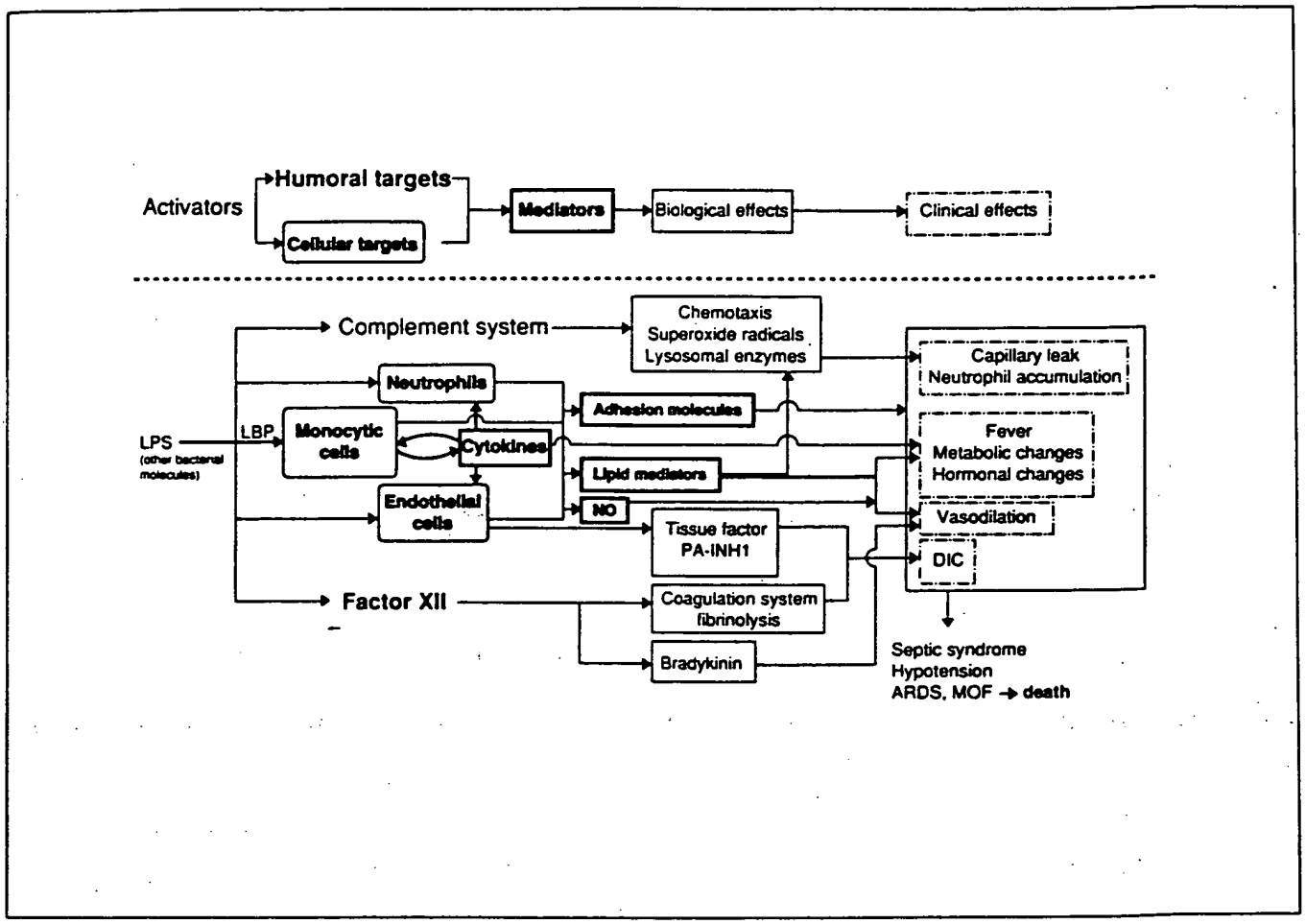

Figure 1. Interaction of humoral factors and cytokines in the pathogenesis of septic shock. LPS = lipopolysaccharide; LBP = LPS-binding protein; PA-INH1 = plasmin activator-inhibitor 1; DIC = disseminated intravascular coagulation; ARDS = adult respiratory distress syndrome; NO = nitric oxide; and MOF = multiple organ failure.

received either placebo or two intravenous doses of E5 (2 mg/kg) 24 hours apart [14]. The two groups of patients were reasonably well matched, although the unavailability of scores on the Acute Physiologic and Chronic Health Examination (APACHE) II for 203 patients represented a potentially important source of bias. Of the 468 evaluable patients, 316 had a documented gram-negative infection; 179 of these 316 patients presented with shock. Among the 137 patients who did not present with shock, treatment with E5 was associated with significantly lower 30-day mortality ($P = .03$) and with significantly more frequent resolution of major morbidities (i.e., complications of shock such as disseminated intravascular coagulation [DIC] and acute renal failure) ($P = .04$) than was placebo administration. However, no differences in mortality were found between E5-treated patients and placebo recipients who presented with shock or between E5-treated patients and placebo recipients who did not have gram-negative sepsis. Administration of E5 was found to be safe; it did cause an increase in the level of antibody to murine immunoglobulin in approximately one-half of patients, but the titer was usually low and of no clinical importance.

Since this study suggested that E5 was effective in a subgroup of patients defined retrospectively, a second multicenter study was conducted to verify the finding prospectively. A total of 847 patients without shock were enrolled. The favorable results of the first study were not confirmed: treatment with E5 did not affect survival among the 530 patients with documented gram-negative sepsis. However, a favorable—albeit statistically insignificant—trend was noted in the subgroup of patients in whom gram-negative sepsis was associated with major morbidities, such as DIC and renal failure [16]. A third trial of E5 is under way.

A clinical trial was also performed with HA-1A, a human monoclonal antibody to lipid A. In a study similar in design to that described for E5, patients with suspected gram-negative infection were randomized to receive either an albumin placebo or a single 100-mg intravenous dose of HA-1A [15].

Of 543 patients enrolled in the study, 317 had microbiologically documented gram-negative infections; 117 of these 317 patients had had sterile blood cultures at randomization. HA-1A did not reduce mortality either in the overall study population or in the 117 patients with nonbacteremic gram-negative infections. However, mortality did decrease with treatment among the 200 patients with gram-negative bacteremia ($P = .014$). While this difference in mortality was also noted among the 101 patients with gram-negative bacteremia who presented with shock at enrollment, no such difference was found among the 99 patients with gram-negative bacteremia who did not present with shock. These data contrast with the findings from the first study of E5, in which patients appeared to be protected whether or not they were bacteremic but only when they were not in shock.

This report of reduced mortality in a subgroup of patients given HA-1A led to the licensure of the product in some European countries. However, in the United States, the Food and Drug Administration did not approve this product for several reasons, including (1) changes in the protocol during the clinical trial; (2) the documentation of significant differences only in subgroups of patients; and (3) possible imbalances favoring the test drug at randomization of the patients [17]. Therefore, a second study that focused on patients with gram-negative bacteremia and shock was initiated. During this trial the manufacturer voluntarily withdrew HA-1A from the market in Europe because of excess mortality in patients without gram-negative bacteremia [18].

These disappointing results with the two monoclonal antibodies to lipid A are, perhaps, not totally unexpected in light of the studies that led to their development. First, in the original experiments testing the hypothesis that cross-protection could be provided by antibodies to core glycolipid of endotoxin, killed bacteria from a rough-cell-wall mutant strain of *Escherichia coli* O111 (strain J5) were used for the immunization of animals or humans in order to obtain polyclonal antisera. Although some studies showed protection with polyclonal antisera to J5, it was impossible to show definitively that protection was attributable to cross-protective antibodies. Indeed, a favorable outcome could not be correlated with antibody titers in either of the two clinical studies done with human polyclonal antisera to J5 ([10, 11] and J. D. Baumgartner, unpublished data). By solubilization of the core glycolipid in a physiological manner to circumvent nonspecific binding [19], the J5 antisera used in one study were later shown to contain IgG and IgM antibody to J5 at a titer only threefold higher than that in control (preimmune) serum; furthermore, these antisera contained no more antibodies to lipid A than did the control serum [20]. Moreover, it now appears that antibodies to J5 are highly specific for *E. coli* J5 and that they do not cross-react with endotoxin from other bacteria. Hence, the mechanisms of protection by antisera to J5 remain unknown. Nevertheless, this approach with polyclonal sera raised against rough structures of LPS laid the groundwork for the development of therapeutic monoclonal antibodies to core glycolipid.

Second, it now appears that clinical trials with these monoclonal antibodies to lipid A were initiated before the immunologic reactivity of these antibodies for LPS substructures was recognized. Since the first disclosure of the possible efficacy of E5 and HA-1A in patients with septic shock [14, 15], many studies have been undertaken to define the reactivity of these antibodies for LPS. In studies by Fujihara et al. [21] and Mascelli et al. [22], HA-1A reportedly bound to lipid A, rough LPS structures, and some preparations of smooth LPS; however, Baumgartner could not confirm such reactivity [23]. Thus the specificity of these antibodies for lipid A is uncertain, in part because immunoglobulins—especially those of the IgM isotype—tend to bind nonspecifically to both the highly amphophilic core oligosaccharide of LPS and lipid A molecules.

It has also been suggested that HA-1A lowers levels of endotoxin by mediating the clearance of LPS via complement-dependent binding of LPS to complement receptor expressed on erythrocytes and neutrophils [24, 25]. Similarly, E5 was shown to bind to lipid A, to rough LPS, and to 15 preparations of smooth LPS isolated from various strains of gram-negative bacteria [26, 27]. However, neither HA-1A nor E5 was able to neutralize LPS in vitro, as assessed by the limulus lysate test, by a mitogenic assay for murine splenocytes, or by measurement of the production of cytokines in human whole blood [28].

Studies with HA-1A in vivo have yielded inconclusive results. Initially, HA-1A was reported to be protective in mice when used unpurified (as hybridoma fluid) [29]; however, a purified monoclonal antibody obtained from the same clone was not protective in similar experiments and did not suppress endotoxin-induced production of TNF in vivo [30]. A similar failure of HA-1A to protect mice from LPS-induced death was recently reported [31]. More important, in recent experiments with dogs, HA-1A did not alter levels of bacteremia or endotoxemia and was actually associated with a decreased rate of survival [32]. E5, initially studied in vivo by Young et al. [33], was subsequently shown to be beneficial in a model of pseudomonas sepsis in neutropenic mice [34].

The discrepancies in these results of in vitro and in vivo studies stressed the need for extensive characterization of antibodies to core glycolipid before the initiation of clinical trials.

Activation of Pathways Other Than Cytokines by Cell Wall Components

Numerous humoral mediators of sepsis have been identified so far, and it is likely that more will be discovered. These mediators act through complex synergistic and antagonistic interactions. LPS in the blood activates the coagulation and complement cascades and induces a broad array of mediators

from macrophages and other cells, including endothelial cells.

The alternative complement pathway can be activated experimentally by LPS and gram-positive bacterial cell-wall components. The classic pathway is activated mainly by complexes of cell wall components and antibodies. The anaphylatoxins C3a and C5a, which are produced as a result of activation of these pathways, are responsible for a series of inflammatory events that have been implicated in the pathophysiology of septic shock. C5a has been directly associated with TNF and LPS in hemorrhagic necrosis [35]. Complement components induce vasodilation and increased vascular permeability, which can result in hemodynamic changes, aggregation of platelets, and aggregation and activation of neutrophils—all processes that have been implicated in the pathogenesis of the adult respiratory distress syndrome [36]. The subsequent release of arachidonic acid derivatives, cytotoxic products of molecular oxygen, and lysosomal enzymes exerts additional local vasoactive effects on the microvasculature and causes endothelial cell cytotoxicity, which results in capillary leakage. An increased concentration of activated complement has been associated with a fatal outcome in septic shock of both gram-positive and gram-negative origin [37].

It is well known that arachidonic acid metabolites cause vasodilation, platelet aggregation, and neutrophil activation, which may contribute to the pathogenesis of septic shock. These substances are found in increased concentrations after experimental challenge with endotoxin and during septic shock in patients [38]. The role of inhibitor/antagonists of the pathways of arachidonic acid metabolism in the treatment of septic shock is being investigated.

Activated neutrophils, a key element in the inflammatory response, probably play an important part in the pathogenesis of septic shock by contributing to vascular and tissue injuries. Strong evidence indicates that neutrophils are activated either directly by LPS or indirectly through the action of cytokines [39]. As a result, activated neutrophils may damage tissues by releasing oxygen metabolites and lysosomal enzymes, or they may cause microemboli after aggregation. Activated leukocytes adhere to one another, to endothelial cells, and to tissues through interactions of receptors (on endothelial cells) and ligands (on inflammatory cells) that are mediated by specific adhesion molecules (figure 2). The adhesion process is essential to most functions of leukocytes (including chemotaxis, phagocytosis, and cytotoxicity [40]), and blocking of the adhesion process by monoclonal antibodies prevents tissue injury and improves the survival rate in animal models of septic shock [41].

Two humoral factors are currently being evaluated for their role as mediators of septic shock: platelet-activating factor (PAF) and nitric oxide (NO). The infusion of endotoxin induces the release of PAF, a potent phospholipid mediator that leads to autocatalytic amplification of cytokine release. PAF is a mediator of inflammation caused by macrophages, neutrophils, platelets, and endothelial cells in response to injury (figure 2). Elevated levels of PAF have been found in models of endotoxin-induced hypotension and endotoxin-induced lung injury in rats [42, 43]. Many antagonists to PAF exist [44]. The first data from a randomized clinical trial of the efficacy of a PAF antagonist in severe sepsis have recently been reported [45]. In this study 262 patients received either placebo or the PAF antagonist. Mortality decreased by 42% with PAF antagonist treatment in a subset of 119 patients with documented gram-negative sepsis (57% vs. 33%; $P = .011$). A confirmatory study focusing on gram-negative sepsis is now in progress.

Hypotension during septic shock may reflect increased synthesis of the potent vasodilator NO. Considerable information is now available on the activity of NO in vitro [46]. Upon LPS challenge, NO is produced mainly by macrophages, endothelial cells, smooth muscle cells, and the liver. Although LPS-induced release of NO by macrophages appears to take several hours, endothelial cells react within minutes—a phenomenon that may contribute to the rapid decrease in blood pressure associated with endotoxic shock [47]. An increasing number of reports deal with the inhibition of this pathway in animal models, but the precise role of NO in septic shock remains unclear [48]. Activity of NO has been detected in experimental gram-negative sepsis [49] and in patients with the sepsis syndrome [50]. While NO blockade in animals has been reported to be beneficial in some studies [51, 52], it has also been found to be detrimental [53]. Treatment with *N*-monomethyl-L-arginine, which blocks NO synthesis, transiently restored blood pressure and systemic vascular resistance in two patients in whom conventional therapy failed [54]. While NO has potentially deleterious effects in endotoxemia, a critical level of locally produced NO is needed to maintain vascular tone. Therefore, more experimental and clinical studies must explore the role of NO in septic shock and delineate the potential for its inhibition in patients.

Factor XII (Hageman factor) in the coagulation cascade has long been known to play a central role in the pathogenesis of septic shock. It is activated equally efficiently by peptidoglycan residues and teichoic acid from the cell wall of gram-positive organisms—e.g., *S. aureus* and streptococci (including pneumococci)—and by LPS and lipid A from gram-negative bacilli [55, 56]. Through activation of Factor XI, activated Factor XII triggers the production of tissue factor both by the intrinsic coagulation pathway and by endothelial cells and macrophages; in turn, tissue factor activates the extrinsic coagulation pathway (figures 1 and 2). The activation of these pathways through these various stimuli may lead to consumption of coagulation factors and DIC. Moreover, the activation of the contact system by LPS-activated Factor XII (i.e., activation of Factor XII when it comes in contact with negatively charged surfaces, such as those of

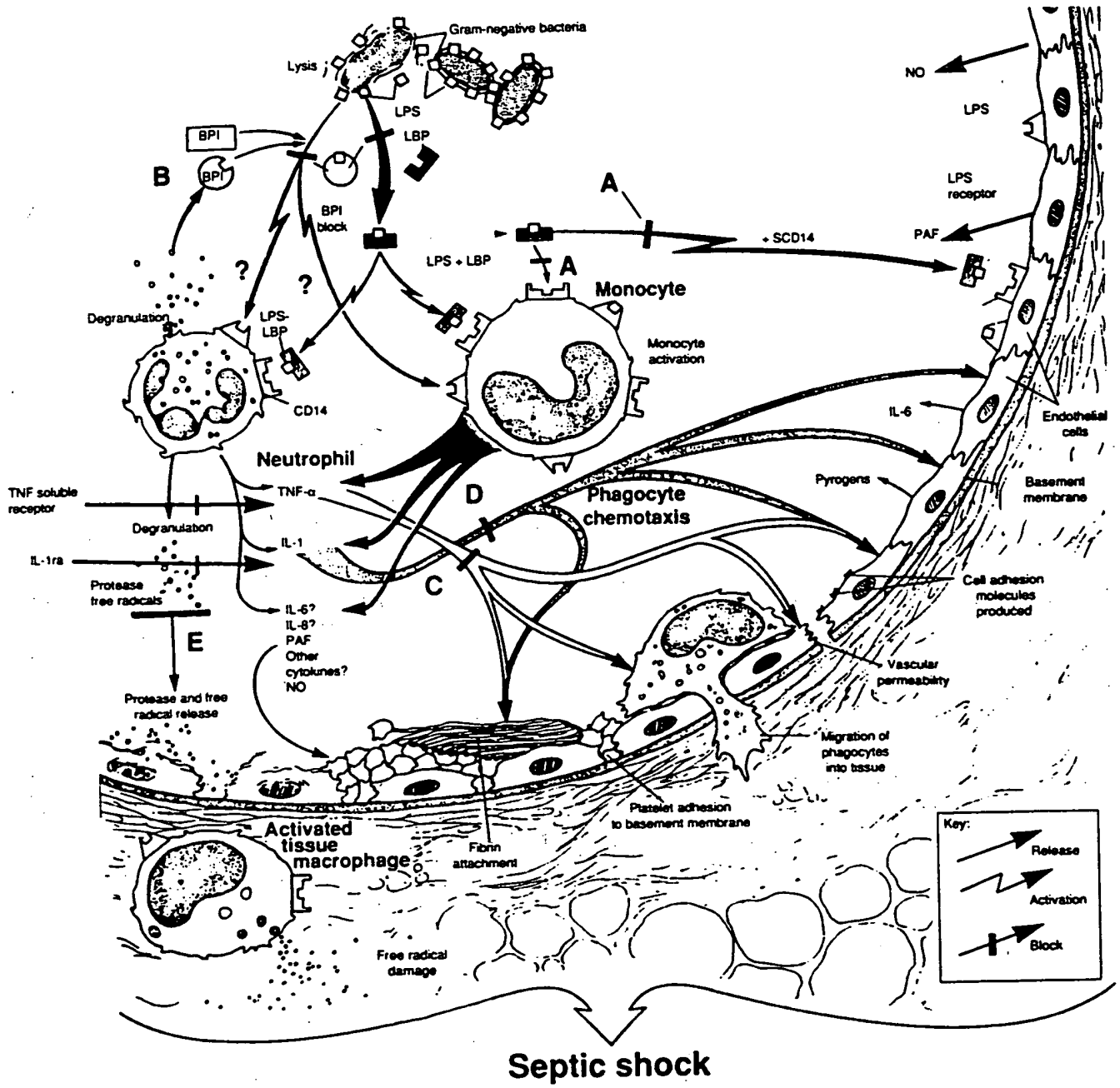

Figure 2. Experimental approaches to blocking septic shock. (*A*) Monoclonal antibodies to LPS prevent LPS from activating inflammatory reactions. (*B*) Bactericidal/permeability-increasing protein (BPI), a protein from neutrophil granules, binds LPS. Neither BPI nor antibodies to LPS function when gram-positive organisms invade the bloodstream. (*C*) TNF-α is inhibited by antibodies or soluble TNF-α receptors. (*D*) IL-1 is blocked by soluble IL-1 receptors or IL-1 receptor antagonist (IL-1ra), a naturally occurring human protein. (*E*) Tissue damage later in the septic shock cascade is minimized by protease inhibitors and free-radical scavengers or agents blocking other cytokines, including IL-6 and IL-8. LBP = LPS-binding protein; PAF = platelet-activating factor; NO = nitric oxide; and SCD14 = soluble CD14.

organisms or substances) results in the conversion of prekallikrein to kallikrein. In turn, kallikrein cleaves high-molecular-weight kininogen to release bradykinin, a potent hypotensive agent [57]. The hypotension and DIC of bacteremia may be mediated—at least in part—by the activation of the contact system via the release of bradykinin and the activation of Factor XI. Recently, a monoclonal antibody to Factor XII was used to block contact activation in baboons; the results showed that the contact system contributes to hypotension but not to DIC in lethal bacteremia [58]. The fact that tissue factor produced upon stimulation of macrophages and endothelial cells by LPS plays a major role in inducing

DIC is indicated by the prevention of LPS-induced DIC by antibody to tissue factor in animals [59, 60]. Evidence also exists that protein C can be activated during gram-negative bacteremia [61] and that activated protein C can prevent the coagulopathic and lethal effects of *E. coli* infusion in baboons [62].

Finally, it is widely recognized that vascular endothelium plays an active role in the development of septic shock. The systemic effects of high doses of intravenously administered TNF or IL-1 into animals include hypotension, decreased systemic vascular resistance, vascular leak, and infiltration of polymorphonuclear leukocytes (PMNs) into tissues [63]. Enhancement of the expression of adhesion molecules on endothelial cells by TNF and IL-1 facilitates PMN margination. TNF and IL-1 increase the procoagulant activity of endothelium and depress the expression of fibrinolytic activity; these changes result in a trend towards intravascular coagulation [64]. Generally, in animals given antibody to TNF or IL-1 receptor antagonist (IL-1ra), an attenuation and/or blockade of many pathophysiological changes is associated with activation of the coagulation cascade mediated by endothelial cells and cytokines [65, 66].

The Cytokine Network

Monocytic cells appear to have a pivotal role in mediation of the biological effects of LPS (figure 2). First, LPS can be removed from the blood and detoxified by monocytes—events of benefit to the host [67]. Second, LPS-stimulated monocytes produce cytokines such as TNF and IL-1. Several binding sites for LPS on the macrophage surface have been described [68]. LPS can also interact with monocytes after binding to plasma molecules. An acute-phase protein called LPS-binding protein (LBP) has been shown to bind to the lipid A moiety of LPS [69]. LPS-LBP is a ligand for the CD14 receptors on monocytes and macrophages [70]. Furthermore, a soluble form of the CD14 receptor in serum has been shown to promote the binding of LPS to endothelial cells and to stimulate these cells to produce cytokines and adhesion molecules [71, 72]. The role played by soluble CD14 in vivo is still unknown. When complexed with LBP and attached to monocytes, LPS can stimulate the production of TNF by macrophages at concentrations far below those required for stimulation by LPS alone [69, 70, 73]. Recently, another active component of human plasma, septin, has been described; septin may share with LBP the capacity to enhance the presentation of low concentrations of LPS to monocytes [74]. This information suggests that recognition of the presence of LPS in plasma is important for an effective response to infection with gram-negative bacteria. Therefore, a principal function of LBP/septin may be to enhance the ability of the host to detect LPS early in infection [69, 70, 74]. No information is available on the role of LBP/septin or CD14 in vivo, but studies of the blocking of LBP or CD14 in experimental models will help to define the early steps of interaction of LPS and monocytes in the development of shock.

Bactericidal/permeability-increasing protein (BPI), a protein that has significant amino acid sequence homology with LBP (figure 2), appears to have therapeutic potential; it is found in PMN azurophilic granules that bind to lipid A and LPS in a way similar to that documented for LBP. BPI is an antagonist of LBP because it inhibits rather than amplifies the activity of LPS. In addition, BPI has been shown to inhibit the limulus lysate assay mediated by LPS or the LPS-induced production of cytokines in blood [75, 76]. Experiments in animals challenged with endotoxin and gram-negative bacteria are now planned with a cloned recombinant protein [77], and preliminary data on BPI-mediated protection of mice challenged with LPS have been presented [78].

The intravascular activation of inflammatory systems involved in septic shock is mainly the consequence of an overproduction of various cytokines. Several cytokines are produced not only by macrophages but also by lymphocytes, endothelial cells, and other cells stimulated by microbial products. The systemic release of large amounts of cytokines is associated with death from septic shock in humans [79–81].

TNF is regarded as a central mediator of the pathophysiological changes associated with the release of LPS and possibly with shock caused by microorganisms that do not contain LPS. In animal models, antibodies to TNF—given either prophylactically (before intravenous bolus injections of LPS or gram-negative bacteria) or therapeutically (after challenge)—have effectively increased the rate of survival. A potentially important advantage of making TNF (rather than endotoxin) a target in intervention strategies in patients is its possible role in the pathogenesis of shock caused by gram-positive bacteria. For example, septicemia associated with group A *Streptococcus* is clinically indistinguishable from "classic" gram-negative septic shock [82]. Cell-free supernatants from cultures of gram-positive bacteria have been shown to induce the release of TNF from human peripheral-blood monocytes in vitro [83], and concentrations of TNF in the serum of patients with gram-positive sepsis are as high as those in the serum of patients with gram-negative sepsis [84]. However, antibodies to TNF have not been universally effective in models of gram-positive sepsis [85–87].

Cytokines other than TNF are involved in the induction of a shock-like state in animals. Considerable interest has also been focused on IL-1 as a mediator of shock and of the associated "acute-phase" responses [65]. Circulating levels of IL-1 are elevated in shock; together with elevated levels of TNF, these increased levels of IL-1 correlate with the severity of disease. Direct proof of the central role of IL-1 in septic shock comes from experiments with animals in which specific blocking of the binding of IL-1 to its cell receptor by

IL-1ra prevented the detrimental effects of inoculation of LPS or *E. coli* [88–90].

The overlap of proinflammatory functions and the synergy of TNF and IL-1 are probably important clues to the pathogenesis of septic shock. The administration of low doses of IL-1 does not mediate severe shock and tissue injury; when IL-1 is combined with TNF, however, the former increases the toxicity of the latter [91]. More important, LPS itself potentiates the lethal effects of TNF [92]. Interferon γ has also been implicated in the synergy of TNF and IL-1 [93]. Since several cytokines are probably involved in the pathogenesis of septic shock, blocking of TNF alone may not be sufficient to reverse the relevant conditions; therefore, pharmacotherapeutic "cocktails" may prove necessary.

The elucidation of these pathophysiological events has prompted the development of strategies to counteract excessive production or release of TNF and IL-1 and hence to prevent or treat septic shock. Many studies have indicated that such therapy is beneficial in animals (including mice, rats, pigs, rabbits, and baboons). However, other important observations regarding potential therapeutic strategies must be considered. First, TNF, IL-1, and other cytokines are released into the bloodstream during the first hour after bolus injection of LPS or live bacteria; TNF disappears rapidly from the circulation thereafter—several hours before the animal's death [94]. An identical pattern has been found in children with fulminant meningococcemia [80]. Should it be documented in most cases of septic shock in humans, this pattern would suggest that levels of TNF may be elevated before shock develops. If so, then perhaps antibodies to TNF could not be administered soon enough to effectively treat patients with full-blown shock.

Moreover, intravenous bolus challenge in animal models and the fulminant course of meningococcal septicemia in children may not reflect most of the clinical situations in which septic shock develops. In most typical cases, a focus of infection may be present for hours or days and may cause the release of LPS or bacteria to be more sustained and subacute than during fulminant shock. Indeed, when serum concentrations of TNF and other cytokines were measured prospectively in 70 patients with septic shock, levels of TNF and IL-1 were rather low and were detectable for up to 10 days after the onset of shock in patients who ultimately died [81]. These results indicate that concentrations of TNF and other cytokines are sustained in patients presenting with gram-negative shock—a picture unlike that seen after LPS or bacterial challenge in animal models, in which these concentrations are elevated and transient. Moreover, experimental models of severe, subacute, focal gram-negative bacterial infection have exhibited a pattern of TNF release different from that observed after bolus injection, and antibodies to TNF have failed to prevent death in these models [95–97]. Thus the release of TNF in most clinical cases of septic shock is probably different from that in fulminant gram-negative infections, and anticytokine therapies should be devised accordingly.

In such anticytokine therapies, the role of cytokines as a defense against infection must be taken into account. TNF, IL-1, and other cytokines participate in the defense of the host; they are mediators that increase natural resistance by, for example, upregulating the cytolytic activity of lymphocytes and the expression of complement receptors, enhancing the oxidative burst of neutrophils, activating macrophages, and stimulating the proliferation of B cells, T cells, and progenitor cells. Indeed, while mice rendered deficient in the 55-kD receptor for TNF became resistant to challenge with endotoxin, they meanwhile became extremely sensitive to infection with *Listeria monocytogenes* [98]. Thus the blocking of cytokines in patients with septic shock for the purpose of counteracting "harmful" concentrations may interfere with the control of infection by physiological concentrations. As a result, the infections causing septic shock may worsen, or the patient may become more susceptible to secondary infections.

Two major clinical studies of the blocking of cytokines are in progress, the first examining a murine antibody to TNF and the second evaluating IL-1ra. Preliminary results indicate that anticytokine reagents may have detrimental effects in subgroups of patients; however, it is not known whether the failure observed in patients not presenting with shock is due specifically to a deleterious effect of these reagents on the host defenses against intracellular pathogens. When further results become available, the cause of the deaths of patients in the various subgroups will be analyzed.

The large-scale clinical trial of murine antibody to TNF was initiated in patients presenting with sepsis syndrome and septic shock. While interim analyses revealed a beneficial (although statistically insignificant) effect of treatment on mortality among patients with septic shock, a deleterious effect was found in some patients with sepsis syndrome [99]—a less classic form of the cascade of events that leads to death from infection. As a result, this study was discontinued, and a new trial of antibody to TNF including only patients with septic shock is being planned.

The results of the phase 3 trial of IL-1ra have recently been presented [100]. This randomized, double-blind, placebo-controlled, multicenter clinical trial included 901 patients with sepsis syndrome and septic shock. At enrollment, patients were randomized to receive either an intravenous loading dose of IL-1ra (100 mg) or placebo, which was followed by a continuous 72-hour intravenous infusion of IL-1ra (1 mg/[kg·h] or 2 mg/[kg·h]) or placebo. Patients were evaluated at 28 days for all-cause mortality. Treatment with IL-1ra did not significantly improve the rate of survival, which was the primary end point of the study. However, an individual patient/risk assessment approach, which took into account the APACHE III classification system and specific risk factors for sepsis, was used to analyze outcome as a sec-

ondary end point [101]. With this new method of risk prediction, treatment with IL-1ra appeared to be beneficial for patients with a mortality risk of >0.24 but not for those with a mortality risk of <0.24.

Both of these studies aimed at inhibiting cytokines. The results suggest that, while this approach may be useful for the most severely affected patients, it is potentially harmful to patients who are less severely affected. Obviously, these findings need to be confirmed, and the subgroup of patients who might benefit from these therapies needs to be precisely delineated. The results of the IL-1ra study suggest that defining the risk of death at the time of intervention—rather than at the time of clinical presentation—may be a simple tool for identifying such patients.

Soluble forms of cytokine receptors offer an alternative approach to the blocking of cytokines. The potential of this approach was shown in experiments and clinical studies with IL-1ra; soluble TNF receptors may also be of value (figure 2). These receptors inhibit the bioactivity of TNF in vivo [102, 103]. Furthermore, it has recently been shown that mice deficient in the 55-kD TNF receptor are resistant to endotoxic shock [98]. Clinical trials with soluble TNF receptors are therefore being planned.

Currently, two other cytokines, IL-8 and IL-10, are being evaluated as possibly important mediators in shock. IL-8 has been characterized primarily as a PMN chemoattractant and a proinflammatory mediator; it has been detected in healthy volunteers after intravenous injection of endotoxin [104] and in patients with gram-negative shock [105]. Its precise role in vivo has not yet been fully elucidated. Recently, the anti-inflammatory IL-10 has been suggested as a candidate for treatment of bacterial sepsis. IL-10 was found to decrease the production of IL-1, IL-6, and TNF in vitro and to suppress cytokines and provide protection in mice challenged with lethal doses of endotoxin [106, 107]. It is interesting that protection was documented when the administration of IL-10 was delayed after LPS challenge—an effect that is hardly evident with antibodies to TNF. To investigate the potential of IL-10 as a candidate for the treatment of bacterial sepsis, these preliminary results in endotoxemia should now be extended to bacterial infections.

Finally, as has been mentioned, the synergy that exists among cytokines (especially TNF and IL-1) and between cytokines and cell wall fragments (mainly TNF and LPS) suggests that a combined approach aimed at blocking various triggers and mediators may have the greatest potential for improving the outcome of septic shock.

Conclusion

Several approaches to the treatment and prevention of septic shock that are now being considered aim to suppress and/or inhibit one or another of a range of cytokines and other inflammatory mediators. However, since the syndrome most likely results from complex interactions involving all these pathways and cytokines, the roles that each mechanism plays in the pathogenesis of septic shock must be delineated. This information will help to identify the subsets of patients who might benefit from the administration of antibodies to endotoxin and cytokines and to ascertain the need for other cytokine inhibitors or anti-inflammatory agents.

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